DOES NOT CIRCULATE
UNIVERSITY OF MICHIGAN
MAR 7 1955
MEDICAL LIBRARY


# The Journal of LABORATORY and CLINICAL MEDICINE

VOL. 45 MARCH, 1955 NO. 3

*The Official Publication of the Central Society for Clinical Research*


## Clinical and Experimental


The Value of Phenylbutazone in the Treatment of Rheumatoid Arthritis as Determined by Clinical Response and by the Serum Protein-Polysaccharide Ratio (PR). R. W. Payne, M.D., M. R. Shetlar, Ph.D., Charles H. Farr, B.S., Arthur A. Hellbaum, Ph.D., M.D., and William K. Ishmael, M.D., Oklahoma City, Okla. ---------- 331

The Effect of Thyroid Hormone Upon the Degradation Rate and Miscible Pool of Radioiodinated Human Serum Albumin in Myxedema. Ernest Schwartz, A.M., M.D., Los Angeles, Calif. ---------- 340

Lack of Effect of Fluorine Ingestion on Uptake of Iodine$^{131}$ by the Thyroid Gland. J. Elliot Levi, M.D., and Hannah E. Silberstein, M.S., Baltimore, Md. ---------- 348

The Use of the Urinary Pigment: Creatinine Ratio as a Measure of Basal Metabolic Rate and Thyroid Activity. Ferrin B. Moreland, Ph.D., and Arthur E. Gurgiolo, M.A., Houston, Tex. ---------- 352

Glycine and Synthesis of Para-Aminohippuric Acid in "Free Anxiety" and Hyperthyroidism. Marc J. Musser, M.D., William P. Deiss, Jr., M.D., and Thomas H. Lorenz, M.D., Madison, Wis. With the Technical Assistance of Maria M. Moore, B.S. ---------- 357

Serum and Liver Lipids in Patients With and Without Liver Disease. Barbara H. Billing, Ph.D., Ruth M. Haslam, M.D., David E. Hein, M.D., Harold J. Conlon, M.D., David L. Hamilton, M.D., Gordon M. Mindrum, M.D., and Leon Schiff, M.D., Ph.D., Cincinnati, Ohio. With the Technical Assistance of Lila Dohm, B.S., and Charlene Starr, B.S. ---------- 363

The Rate of Disappearance of Rb$^{86}$ From the Plasma, the Biologic Decay Rates of Rb$^{86}$, and the Applicability of Rb$^{86}$ as a Tracer of Potassium in Man With and Without Chronic Congestive Heart Failure. G. E. Burch, M.D., S. A. Threefoot, M.D., and C. T. Ray, M.D., New Orleans, La. ---------- 371

Study of the Use of Rb$^{86}$ as a Tracer for the Measurement of Rb$^{86}$ and K$^{39}$ Space and Mass in Intact Man With and Without Congestive Heart Failure. S. A. Threefoot, M.D., C. T. Ray, M.D., and G. E. Burch, M.D., New Orleans, La. ---------- 395

The Excretion of Radiorubidium, Rb$^{86}$, Radiopotassium, K$^{42}$, and Potassium, Sodium, and Chloride by Man With and Without Congestive Heart Failure. C. T. Ray, M.D., S. A. Threefoot, M.D., and G. E. Burch, M.D., New Orleans, La. ---------- 408

The Antihypertensive Properties of Some Mercaptans and Other Sulfur-Containing Compounds. Henry A. Schroeder, M.D., Edna M. Menhard, B.S., and H. Mitchell Perry, Jr., M.D., St. Louis, Mo. With the Technical Assistance of Evie G. Dennis 431


*(Contents continued on inside front cover)*

PUBLISHED BY THE C. V. MOSBY COMPANY, ST. LOUIS 3, MISSOURI

Copyright 1955 by The C. V. Mosby Company

## Contents—*(Continued from front cover)*


Further Studies of a Hemostatic Defect Caused by Intravenous Dextran. Edward Adelson, Captain, MC, William H. Crosby, Lieutenant Colonel, MC, and William H. Roeder, Corporal, AMEDS, Washington, D. C. With the Technical Assistance of Carol E. Thibeault ---- 441

Delayed Hypersensitivity in Experimental Fungus Infections. James E. Dyson, Jr., M.S., and E. Edward Evans, Ph.D., Ann Arbor, Mich. ---- 449

The Effectiveness of Antibacterial Drugs Upon the Gram-Negative Intestinal Flora. J. V. Martins Campos, M.D., J. Fernandes Pontes, M.D., W. Hoenen, M.D., and N. Kusminski, M.D., São Paulo, Brazil ---- 455

The Erythrocyte Potassium Concentration in Conditions of Hyponatremia and Hypernatremia. Harvey C. Knowles, Jr., M.D., Gabriele Alverson, and Edward Rubenstein, M.D., Cincinnati, Ohio ---- 463

Lifespan of Fetal Erythrocytes. James W. Hollingsworth, Captain, MC, USA, Washington, D. C. ---- 469

Urinary Vitamin $B_{12}$ Activity Following Intranasal Administration. Raymond W. Monto, M.D., and James T. Howell, M.D., Detroit, Mich. ---- 474

Probenecid and Salicylates: The Question of Interaction in Terms of Penicillin Excretion. William P. Boger, M.D., S. Clyde Strickland, M.D., Gilbert M. Bayne, M.D., and Julina Gylfe, M.D., Norristown, Pa. ---- 478

Effect of Graded Amounts of Epinephrine on Normal and Stress Lymphocytes of Intact and Adrenalectomized Mice. Jules A. Frank, M.D., and Thomas F. Dougherty, Ph.D., Salt Lake City, Utah ---- 485


### Laboratory Methods


A Cardio-Roentgen Actuator: An Instrument for Actuating the Roentgenoscope in End Systole or End Diastole of the Heart Action. Frank W. Noble, M.E.E., Bert R. Boone, M.D., Harold T. Dodge, M.D., and Armand E. Brodeur, M.D., Bethesda, Md. ---- 489

An Anesthesia-Bronchospirometer Valve. Paul O. Anderson, M.D., and John J. Cincotti, M.D., Rutland Heights, Mass. ---- 493

Simple Apparatus for Concentrating Biologic Fluids of Low Protein Content. Charles H. Grogan, Ph.D., and Elizabeth Roboz, Ph.D., Washington, D. C. ---- 495



Editor

**WILLIAM D. ROBINSON, M.D.**

Associate Editor

**WILLIAM H. BEIERWALTES, M.D.**

University Hospital
University of Michigan Medical School
1313 Ann Street, Ann Arbor, Michigan

## Board of Editors

S. HOWARD ARMSTRONG, JR., M.D.
University of Illinois, Chicago

FRANK H. BETHELL, M.D.
University of Michigan, Ann Arbor

RICHARD B. CAPPS, M.D.
Northwestern University Medical School, Chicago

A. C. CORCORAN, M.D.
Cleveland Clinic, Cleveland

ELMER L. DeGOWIN, M.D.
State University of Iowa, Iowa City

RICHARD V. EBERT, M.D.
University of Arkansas, Little Rock

WENDELL H. HALL, M.D.
University of Minnesota, Minneapolis

LEON O. JACOBSON, M.D.
University of Chicago, Chicago

OLIVER H. LOWRY, M.D.
Washington University, St. Louis

W. A. SODEMAN, M.D.
University of Missouri, Columbia

RANDALL G. SPRAGUE, M.D.
Mayo Clinic, Rochester

RICHARD W. VILTER, M.D.
University of Cincinnati, Cincinnati



Vol. 45, No. 3, March, 1955. The Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription price: United States and its Possessions $12.00 per year; Canada $12.50; Foreign $13.50. Subscriptions to medical students, interns, and residents, half-price. Entered as Second Class Matter October 8, 1915, at the Post Office at St. Louis, Missouri, under Act of March 3, 1879. Printed in the U. S. A.

# The Journal of Laboratory and Clinical Medicine

VOL. 45 MARCH, 1955 NO. 3


## CLINICAL AND EXPERIMENTAL

### THE VALUE OF PHENYLBUTAZONE IN THE TREATMENT OF RHEUMATOID ARTHRITIS AS DETERMINED BY CLINICAL RESPONSE AND BY THE SERUM PROTEIN-POLYSACCHARIDE RATIO (PR)


R. W. PAYNE, M.D., M. R. SHETLAR, PH.D., CHARLES H. FARR, B.S., ARTHUR A. HELLBAUM, PH.D., M.D., AND WILLIAM K. ISHMAEL, M.D.

OKLAHOMA CITY, OKLA.


PHENYLBUTAZONE (Butazolidin) has provided symptomatic relief of the articular phenomena of gout, rheumatoid arthritis, and rheumatoid spondylitis in a sufficient number of patients to warrant further objective appraisal of its value. Lack of influence on the sedimentation rate, the traditional index of rheumatic activity, coupled with initial reports of a high incidence of toxicity, has handicapped general acceptance of this drug. Thus, therapeutic evaluation of phenylbutazone would best await evidence of its objective value in the rheumatic diseases, as well as the incidence and severity of undesirable effects which might be expected to attend its use. Despite the lack of significant changes in the sedimentation rate upon administration of phenylbutazone in rheumatoid arthritis,[1, 2] there is evidence of a singular anti-inflammatory action of the drug when compared to other pyrazol derivative analgesics (Currie, Brown and Will[3]). Mason[4] has shown that this effect is probably not due to adrenal stimulation as there is no increase in excretion of 17-ketosteroids or glucocorticoids after administration of phenylbutazone. Furthermore, Kuzell,[5] in a carefully planned study, could show no evidence of adrenal stimulation due to phenylbutazone in either intact or hypophysectomized rats.


From the Departments of Pharmacology, Biochemistry, and Medicine, University of Oklahoma School of Medicine, and the Research Laboratories, V.A. Hospital, Oklahoma City, Okla.



Supported in part by funds made available to the Oklahoma Arthritis and Rheumatism Foundation by the Oklahoma City Community Fund.

Received for publication Oct. 25, 1954.

The serum protein-polysaccharide ratio (PR) has been found to be elevated in rheumatoid arthritis as well as in certain other collagen disorders (Shetlar and associates[6]). This serum component has further been shown to reflect the degree of rheumatoid activity (Payne and co-workers[7]) and, therefore, may serve as an indicator of fundamental changes incidental to the rheumatoid process. The PR may then provide data of objective value in assessing therapeutic measures for these diseases.

The influence of phenylbutazone on the protein-polysaccharide ratio in rheumatoid arthritis is herein described and compared to a similar effect of cortisone and corticotropin.

## METHODS

Patients were followed in the Arthritis Clinic of University Hospitals, the Arthritis Section of the Bone and Joint Hospital, and the V. A. Hospital, all of Oklahoma City. Serum protein-polysaccharide concentrations were determined by the tryptophane method of Shetlar and collaborators.[8] Serum protein was determined by the biuret reaction as described by Weichselbaum.[9] The protein-polysaccharide ratio (PR) was calculated by dividing the serum polysaccharide by the protein and multiplying by 100.

## RESULTS

In Table I data from 82 treatment periods of phenylbutazone (61 patients) are summarized. Daily oral administration of 200 to 600 mg. of phenylbutazone usually produced a significant fall in the elevated PR of patients with rheumatoid arthritis similar to that produced by the oral administration of 100 to 200 mg. of cortisone acetate daily in divided doses. Lesser doses of both drugs, although frequently producing minor degrees of symptomatic relief, were without consistent effect on the PR. These changes in PR paralleled the clinical impression of rheumatoid activity as estimated by the criteria of Steinbrocker and colleagues[10] (Tables II and III). The therapeutic trial of cortisone acetate was generally much shorter than that with phenylbutazone. The sedimentation rate proved of relatively little aid in the present series, reflecting only very gross changes in the disease state. Since results presented in previous publications[6, 7] show no significant correlation between the sedimentation rate and the PR, the former data are not included in the present report.

Although active rheumatoid spondylitis without peripheral joint involvement does not ordinarily produce a high PR, there was a fall in the initial

TABLE I. EFFECT OF CORTISONE ACETATE AND PHENYLBUTAZONE ON CLINICAL ACTIVITY AND PR IN PATIENTS WITH RHEUMATOID ARTHRITIS

| DRUG | DAILY DOSE | TOTAL NUMBER OF TREATMENT PERIODS | CLINICAL IMPROVEMENT GRADE I OR GRADE II | SIGNIFICANT PR FALL |
|---|---|---|---|---|
| Cortisone acetate | 50-100 | 21 | 9 | 8 |
| Cortisone acetate | 101-200 | 9 | 6 | 7 |
| Phenylbutazone | 50-100 | 14 | 5 | 5 |
| Phenylbutazone | 200-300 | 39 | 25 | 27 |
| Phenylbutazone | 301-400 | 27 | 23 | 24 |
| Phenylbutazone | 401-600 | 2 | 2 | 2 |

PR of three patients with this disease after administration of phenylbutazone (Cases 37, 43 and 246) (Table III).

Acute lupus erythematosus is usually accompanied by an exceptionally high PR.[6] One such patient treated with phenylbutazone, 400 mg. per day after an initial course of cortisone, showed clinical improvement paralleled by a drop in PR to a normal value, but elevation accompanied clinical exacerbation after the drug was discontinued due to a skin rash. One case of tophaceous gouty arthritis with persistent activity for a period of two months showed an excellent response to phenylbutazone both clinically and in so far as PR depression was concerned. No detailed analysis of the untoward effects of phenylbutazone will be presented in this series as this matter has been reported.[12, 13] However, this factor prevented the maintenance of adequate therapeutic levels in approximately 14 per cent of these patients No persistent toxic effects were seen after discontinuance of the drug. Patients with cardiovascular disease, previous history of upper GI disturbances, and dermatitis were not given phenylbutazone.

Phenylbutazone dosage was reduced as rapidly as was considered practical to maintain remission with a minimum of side effects. As can be seen (Table III), doses below 200 mg. daily were of only occasional value in control of the rheumatoid process in so far as could be determined by the methods used.

The PR generally falls slowly after adequate phenylbutazone (and cortisone) therapy, requiring 10 days to 2 weeks to show significant changes. Those patients with mild or moderate degrees of rheumatoid arthritis and with minimal deformity responded more rapidly to phenylbutazone, as has been observed clinically by Steinbrocker.[11] Upon discontinuance of phenylbutazone therapy there was usually prompt exacerbation of clinical rheumatoid activity and a rise in the PR. Indeed, a rise in PR frequently heralds an exacerbation of the disease.

## DISCUSSION

As with any antirheumatic drug, the following questions might be applied to phenylbutazone: (a) Is the drug truly effective and does the evaluation of its effectiveness take into consideration such matters as the natural vagaries of the rheumatic diseases, the enthusiasm of the physician, and the concurrent use of general supportive measures? (b) Can the effectiveness of the drug be demonstrated by objective means? (c) Can an effective dose be given without producing disturbing side effects? (d) Does the drug produce toxic effects which persist after the drug has been withdrawn? (e) After initial improvement, can the remission of the arthritic process be maintained over a significant period of time; or, is the ultimate course of the disease altered?

Many observers agree that phenylbutazone is highly effective in symptomatic relief of the articular phenomena of rheumatoid arthritis, rheumatoid spondylitis, and gout. This fact is borne out by the favorable response after

TABLE II. SUMMARY OF CLINICAL AND PR CHANGES IN PATIENTS WITH ARTHRITIC DISEASES TREATED WITH CORTISONE ACETATE AND CORTICOTROPIN

| CASE | SEX | AGE | MG./DAY CORTISONE ACETATE | ORALLY TIME (DAYS) | CORTICOTROPIN | TIME (DAYS) | PR | | CLASSIFICATION* | | ACTIVITY* (GRADE) | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | BEFORE | AFTER | STAGE | CLASS | | |
| 1 | F | 25 | 100 | 14 | | | 1.98 | 1.76 | I | II | I | |
| 2 | M | 43 | 100 | 27 | | | 2.74 | 2.32 | III | III | II | |
| 3 | F | 56 | 100 | 7 | | | 2.65 | 3.14 | III | III | III | |
| | | | 75 | 7 | | | 3.14 | 3.13 | | | III | |
| 4 | F | 56 | | | Aqueous, 20 mg./Q 6 hr. S.C. | 14 | 2.62 | 1.96 | III | IV | I | |
| | | | | | 0 | 14 | 1.96 | 2.19 | | | IV | |
| | | | 100 | 24 | | | 2.19 | 2.53 | | | III | |
| 5 | F | 30 | 100 | 7 | | | 1.93 | 2.03 | I | II | I | |
| | | | 100 | 21 | | | 2.03 | 1.54 | | | I | |
| 6 | F | 48 | 100 | 14 | | | 2.71 | 2.41 | III | III | II | Still's disease |
| 7 | F | 44 | 200 | 7 | | | 2.30 | 2.55 | | | II | Lupus erythematosus |
| | | | 100 | 7 | | | 2.55 | 2.09 | | | I | |
| 8 | F | 55 | 100 | 7 | | | 2.07 | 1.86 | I | II | I | |
| | | | 50 | 30 | | | 1.86 | 1.99 | | | IV | |
| 9 | F | 68 | 100 | 7 | | | 2.51 | 2.45 | III | IV | III | |
| 10 | M | 48 | 100 | 14 | | | 2.86 | 2.50 | III | IV | II | |
| 11 | F | 17 | 100 | 14 | | | 2.91 | 2.97 | III | IV | IV | |
| 12 | M | 57 | 100 | 14 | | | 2.48 | 2.41 | II | II | II | |
| 13 | F | 55 | | | 20 units I.V./day | | 3.82 | 2.49 | | | I | Lupus erythematosus |
| 14 | F | 55 | | | Acthar Gel 40 units I.V./day | 10 | 2.38 | 2.01 | III | II | I | |
| 15 | F | 36 | 50 | 210 | | | 2.09 | 2.48 | | | IV | |
| 16 | F | 19 | | | 20 units I.V./day | 9 | 3.71 | 2.64 | | | II | Lupus erythematosus |
| | | | 300 | 7 | | | 2.64 | 2.47 | | | I | |
| | | | 0 | | | | 2.47 | 3.16 | | | IV | |
| 17 | M | 52 | 75 | 14 | + Neocylate 4 Tabs. | | 3.25 | 2.33 | | | II | |
| | | | | 14 | I.V. daily | | 2.33 | 2.83 | | | IV | |
| 18 | M | 26 | 200 | 11 | | | 3.12 | 2.71 | IV | IV | III | |
| | | | 100 | 14 | | | 2.71 | 2.72 | | | IV | |
| | | | 50 | 30 | | | 2.72 | 3.46 | | | IV | |
| | | | 100 | 14 | | | 2.84 | 2.88 | | | III | |
| | | | 50 | 14 | | | 2.88 | 2.98 | | | III | |
| | | | 200 | 7 | | | 2.88 | 2.88 | | | II | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 150 | 7 | | | 2.88 | 2.43 | | | II | |
| | | | 100 | 14 | | | 2.43 | 2.76 | | | III | |
| | | | 80† | 30 | | | 2.76 | 2.71 | | | II | |
| 19 | F | 67 | 100 | 14 | | | 2.53 | 2.67 | IV | IV | IV | |
| 20 | M | 44 | 100 | 21 | | | 2.54 | 2.40 | II | III | II | |
| 21 | M | 25 | 100 | 16 | | | 3.52 | 3.33 | IV | IV | III | |
| | | | 200 | 7 | | | 3.84 | 3.92 | | | IV | |
| | | | 100 | 14 | | | 3.92 | 3.82 | | | III | |
| | | | 50 | 14 | + Phenylbutazone 400 mg./day<br>Acthar Gel 40 units QOD<br>+ Phenylbutazone 200 mg./day | 71 | 3.82<br>3.37 | 3.37<br>2.17 | | | III<br>I | |
| 22 | M | 56 | 100 | 7 | | | 3.16 | 2.99 | IV | IV | II | |
| | | | 100 | 7 | | | 2.99 | 2.62 | | | II | |
| | | | 50 | 7 | | | 2.62 | 3.00 | | | IV | |
| 23 | M | 58 | 100 | 14 | | | 3.32 | 3.46 | IV | IV | IV | Psoriatic arthritis, severe |
| | | | 125 | 7 | | | 3.46 | 2.86 | | | III | |
| | | | 200 | 7 | | | 2.86 | 2.59 | | | II | |
| | | | 100 | 21 | | | 2.59 | 3.29 | | | IV | |
| | | | 125 | 14 | | | 3.29 | 2.98 | | | III | |
| 24 | M | 22 | 200 | 7 | | | 2.21 | 2.42 | II | III | IV | |
| | | | 100 | 7 | | | 2.42 | 2.08 | | | II | |
| | | | | | Acthar Gel 40 units QOD | 14 | 2.08 | 2.60 | | | IV | |
| | | | 125 | 14 | | | 2.42 | 2.50 | | | III | |

*Estimated by the criteria of Steinbrocker and associates.[10]
†Hydrocortisone instead of cortisone.

TABLE III. SUMMARY OF CLINICAL AND PR CHANGES WITH ARTHRITIC DISEASES TREATED WITH PHENYLBUTAZONE

| CASE | SEX | AGE | MG./DAY | TIME (DAYS) | PR | | CLASSIFICATION* | | ACTIVITY GRADE* | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | BEFORE | AFTER | STAGE | CLASS | | |
| 1 | M | 50 | 600 | 27 | 2.94 | 2.49 | IV | III | II | Hematuria† |
| | | | 300 | 30 | 2.49 | 2.85 | | | IV | |
| 2 | M | 47 | 400 | 14 | 2.53 | 2.33 | II | II | II | |
| | | | 200 | 26 | 2.33 | 2.20 | | | I | |
| 3 | M | 26 | 400 | 30 | 2.93 | 2.57 | IV | IV | II | |
| | | | 400 | 30 | 2.98 | 2.88 | | | III | |
| 4 | F | 56 | 400 | 270 | 2.50 | 1.97 | III | III | I | |
| | | | 200 | 58 | 1.97 | 2.65 | | | IV | |
| 5 | M | 52 | 400 | 18 | 2.83 | 2.50 | II | II | II | |
| | | | 200 | 30 | 2.50 | 2.51 | | | II | |
| 6 | F | 47 | 400 | 180 | 2.37 | 1.77 | III | III | I | |
| | | | 200 | 120 | 1.77 | 2.47 | | | IV | |
| 7 | M | 44 | 400 | 28 | 2.49 | 1.97 | III | III | I | |
| | | | 200 | 60 | 2.15 | 2.32 | | | IV | |
| 8 | F | 37 | 400 | 28 | 1.71 | 1.72 | I | II | III | |
| 9 | M | 61 | 400 | 30 | 2.31 | 2.07 | II | II | I | |
| 10 | F | 37 | 400 | 250 | 2.23 | 1.57 | III | II | III | |
| 11 | F | 46 | 400 | 180 | 2.62 | 2.00 | III | IV | I | GI disturbance† |
| | | | 200 | 90 | 2.00 | 2.28 | | | IV | |
| 12 | M | 34 | 400 | 240 | 2.41 | 1.83 | I | II | I | |
| 13 | M | 22 | 400 | 14 | 2.60 | 2.42 | III | III | II | |
| 14 | F | 30 | 400 | 14 | 1.54 | 2.18 | I | II | IV | Anemia† |
| 15 | M | 25 | 400 | 120 | 2.05 | 1.83 | I | II | I | |
| 16 | M | 30 | 400 | 60 | 3.56 | 2.97 | III | III | II | |
| 17 | M | 39 | 400 | 14 | 2.03 | 1.88 | I | II | II | Skin rash† |
| 18 | M | 34 | 400 | 14 | 2.79 | 2.39 | III | III | II | |
| | | | 100 | 62 | 2.39 | 2.38 | | | IV | |
| | | | 200 | 90 | 2.38 | 2.19 | | | I | |
| | | | 100 | 89 | 2.19 | 2.51 | | | III | |
| 19 | F | 74 | 400 | 330 | 2.81 | 2.34 | III | III | III | |
| 20 | F | 44 | 400 | 54 | 2.68 | 2.30 | | | I | Lupus erythematosus |
| | | | 400 | 65 | 2.09 | 1.89 | | | I | |
| | | | 200/wk. | 60 | 1.89 | 2.17 | | | IV | |
| | | | 200/wk. | 60 | 2.16 | 1.94 | | | I | + Cortisone 25 mg. daily |
| 21 | F | 24 | 400 | 90 | 2.18 | 1.91 | III | III | II | Still's disease |
| | | | 100 | 90 | 1.91 | 1.46 | | | I | |
| | | | 0 | 7 | 1.46 | 2.19 | | | IV | |
| 22 | M | 50 | 400 | 55 | 2.41 | 2.12 | III | II | II | |
| | | | 200 | 240 | 2.12 | 1.54 | | | I | |
| | | | 0 | 14 | 1.54 | 1.91 | | | IV | |
| 23 | F | 58 | 400 | 120 | 2.40 | 2.05 | III | III | II | |
| 24 | M | 40 | 400 | 153 | 2.28 | 2.12 | II | II | I | |
| 25 | F | 36 | 400 | 120 | 2.22 | 2.06 | I | II | I | Anemia† |
| | | | 0 | 180 | 2.06 | 2.48 | | | IV | Anemia† |
| 26 | F | 61 | 300 | 39 | 2.31 | 2.41 | II | II | III | |
| | | | 500 | 67 | 2.41 | 1.90 | | | I | |
| 27 | M | 27 | 300 | 30 | 2.56 | 2.25 | II | II | III | |

*Estimated by the criteria of Steinbrocker and associates.[10]
†Phenylbutazone discontinued.

TABLE III—CONT'D

| CASE | SEX | AGE | MG./DAY | TIME (DAYS) | PR | | CLASSIFICATION* | | ACTIVITY GRADE* | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | BEFORE | AFTER | STAGE | CLASS | | |
| 28 | F | 17 | 300 | 363 | 3.40 | 2.72 | III | III | II | |
| | | | 200 | 90 | 2.72 | 2.91 | | | IV | |
| 29 | M | 63 | 300 | 35 | 2.80 | 2.39 | II | III | II | GI upset† |
| 30 | M | 57 | 300 | 25 | 2.62 | 2.76 | III | III | III | |
| | | | 400 | 14 | 2.76 | 2.65 | | | II | |
| 31 | M | 43 | 200 | 60 | 2.49 | 2.59 | III | III | IV | |
| 32 | M | 12 | 200 | 28 | 2.78 | 2.25 | III | III | II | Still's disease |
| | | | 0 | 70 | 2.25 | 2.74 | | | IV | |
| 33 | F | 64 | 200 | 180 | 2.34 | 1.50 | II | II | I | |
| | | | 0 | 90 | 1.50 | 2.07 | | | IV | |
| 34 | M | 54 | 200 | 57 | 2.22 | 2.02 | I | II | IV | No subjective improvement |
| | | | Placebo | 37 | 2.02 | 2.42 | | | IV | |
| 35 | F | 16 | 200 | 114 | 3.33 | 3.11 | IV | IV | III | |
| 36 | M | 43 | 200 | 28 | 2.18 | 2.11 | I | II | I | |
| 37 | M | 49 | 200 | 76 | 2.08 | 1.68 | III | III | I | Rheumatoid spondylitis |
| 38 | F | 53 | 200 | 30 | 2.15 | 2.45 | I | II | I | CA of colon on operation |
| 39 | F | 68 | 200 | 150 | 3.43 | 2.28 | II | III | I | |
| 40 | M | 50 | 200 | 8 | 2.30 | 2.10 | | | I | Pretophaceous gout |
| 41 | M | 52 | 200 | 90 | 2.40 | 2.24 | I | II | II | |
| 42 | F | 50 | 200 | 90 | 2.22 | 1.96 | I | II | I | Psoriasis: unimproved |
| 43 | M | 50 | 200 | 30 | 1.88 | 1.76 | III | III | III | Rheumatoid spondylitis |
| 44 | M | 36 | 200 | 120 | 2.64 | 2.23 | III | III | II | |
| 45 | F | 45 | 200 | 300 | 2.11 | 1.90 | I | II | I | |
| 46 | F | 22 | 200 | 60 | 1.69 | 1.42 | II | II | II | Rheumatoid spondylitis |
| 47 | F | 32 | 200 | 180 | 2.41 | 2.00 | II | III | II | |
| 48 | M | 40 | 200 | 300 | 2.73 | 2.35 | III | II | II | |
| 49 | F | 45 | 200 | 90 | 2.02 | 1.91 | I | II | I | |
| | | | 0 | 90 | 1.91 | 2.14 | | | IV | Edema† |
| 50 | F | 64 | 200 | 150 | 2.26 | 1.97 | II | II | I | |
| 51 | F | 64 | 300 | 14 | 2.43 | 2.09 | III | III | I | |
| | | | 100 | 60 | 2.09 | 2.43 | | | IV | |
| 52 | M | 54 | 200 | 181 | 2.28 | 1.87 | I | II | I | |
| 53 | F | 62 | 100 | 28 | 2.70 | 2.98 | II | II | IV | |
| | | | 300 | 28 | 2.98 | 2.53 | | | II | |
| 54 | M | 50 | 100 | 126 | 2.42 | 2.40 | I | II | III | |
| 55 | F | 8 | 100 | 28 | 2.17 | 2.50 | III | III | II | Jaundice† |
| 56 | F | 76 | 100 | 90 | 2.49 | 1.84 | I | II | I | |
| 57 | M | 50 | 100 | 90 | 3.10 | 2.86 | III | III | III | |
| | | | 200 | 28 | 2.86 | 2.59 | | | II | |
| | | | 200 | 180 | 2.82 | 2.71 | | | III | |
| 58 | F | 48 | 100 | 60 | 2.23 | 2.28 | I | II | II | |
| 59 | F | 61 | 100 | 210 | 2.39 | 2.25 | II | II | II | |
| | | | 0 | 90 | 2.25 | 2.48 | | | IV | |
| 60 | F | 45 | 50 | 45 | 2.55 | 2.70 | II | III | IV | Nausea† |
| 61 | F | 30 | 100 | 180 | 2.18 | 2.42 | I | II | IV | |

*Estimated by the criteria of Steinbrocker and associates.[10]
†Phenylbutazone discontinued.

administration of the drug as well as by return of symptoms following its withdrawal or decrease of dose below an effective level. Placebo-controlled studies of the drug have been conducted without detracting from this opinion.[12] Clinical course of the patients was independently evaluated by the three clinical members of the investigating team. The PR values were made known to the clinical members in the latter portion of the study as an aid in arriving at the minimal effective dose for each patient. An effort was made to limit medication to the single drug under study, and patients were questioned at each visit as to previous medication. It is realized, however, that many patients with rheumatoid arthritis have an extensive personal armamentarium from which they are prone to supplement the prescribed therapy unless under a rigidly supervised program.

As previously reported, the PR serves as a useful index of clinical activity in rheumatoid arthritis.[7] Further evidence is here presented that a similar situation exists in gout and lupus erythematosus.

In the production of significant changes in the PR phenylbutazone compares favorably with cortisone and corticotropin in rheumatoid arthritis, rheumatoid spondylitis, gout, and perhaps lupus erythematosus.

The frequent incidence of toxic reactions to phenylbutazone, although usually mild, constitutes a considerable handicap, even to conservative use of the drug. Thus, in 14 per cent of the cases here presented discontinuance of the drug was considered necessary due to undesirable side reactions, an incidence in agreement with the figure of 15 per cent reported by Kuzell and associates.[5] A higher incidence of toxic effect (20 per cent) has been reported by Brodie and co-workers,[12] using higher doses of the drug (800 mg. per day). Although no clear brief can be presented at this time as to whether phenylbutazone will become a standard agent for treatment of the rheumatoid diseases, the present evidence would indicate that it is of objective value in suppressing fundamentally harmful processes accompanying rheumatoid arthritis with a reasonable factor of safety.

No persistent toxic effects have been observed after withdrawal of the drug in the series here presented. This is in agreement with the extensive study of Kuzell,[5] although several deaths have been reported concurrent with phenylbutazone administration.[13] Whether the beneficial therapeutic response to phenylbutazone represents alteration of the course of the rheumatic diseases or merely suppression of symptoms must await more prolonged studies, although our present data would seem to indicate the latter concept. The natural variability of the clinical course of rheumatoid arthritis compels a most conservative attitude concerning the evaluation of any therapy. It would appear, however, that any suppression of inflammatory activity should be eagerly sought from the standpoint of relief of subjective distress and to the end of minimizing crippling which might result from this inflammation.

The similarities of the phenylbutazone and cortisone effects on the PR and the dichotomy of their effect on sedimentation rate and possible mode of action are of extreme interest.

Upon evidence presented it would appear that in many patients with rheumatic diseases an effective dose of phenylbutazone can be prescribed with a reasonable factor of safety. It is felt that the PR has served as a valuable aid in determining the minimal effective dose of phenylbutazone and in demonstrating its objective value in the treatment of rheumatoid arthritis.

## SUMMARY

Phenylbutazone (Butazolidin) in doses of 200 to 600 mg. daily has been found to produce a fall in the serum protein-polysaccharide level (PR) in 53 of 68 trials in patients with rheumatoid arthritis. Oral cortisone acetate in doses of 100 to 200 mg. daily produces a similar effect. The fall in PR follows closely the clinical estimation of rheumatoid activity and serves as an objective measure of the latter.

This has led to the conclusion that phenylbutazone produces more than a simple analgesic effect in rheumatoid arthritis.

## REFERENCES

1. Davies, H. R., Barter, R. W., Gee, A., and Hirson, C.: Observations on the Use of "Butazolidin" in Arthritis, Brit. M. J. **2:** 1392-1394, 1952.
2. Hemming, A., and Kuzell, W. C.: The Pharmacologic and Clinical Characteristics of Phenylbutazone (Butazolidin), Antibiotics & Chemother. **3:** 634-644, 1953.
3. Currie, J. P., Brown, R. A. P., and Will, G.: Observations on the Treatment of Rheumatoid Arthritis With Butazolidin, Ann. Rheumat. Dis. **12:** 88-94, 1953.
4. Mason, R. M.: Comparative Effects of ACTH and Butazolidin in Rheumatoid Arthritis, Ann. Rheumat. Dis. **12:** 82-87, 1953.
5. Kuzell, W. C., Schaffarzick, R. W., Brown, B., and Mankle, E. A.: Phenylbutazone (Butazolidin$^R$) in Rheumatoid Arthritis and Gout, J. A. M. A. **149:** 729-734, 1952.
6. Shetlar, M. R., Payne, R. W., Bullock, Jane Ann, Patrick, D. R., Hellbaum, A. A., and Ishmael, W. K.: Comparative Studies of Serum Polysaccharides in Rheumatoid Arthritis and Degenerative Joint Diseases, J. Clin. Invest. **32:** 1208-1213, 1953.
7. Payne, R. W., Shetlar, M. R., Bullock, Jane Ann. Patrick, D. R., Hellbaum, A. A., and Ishmael, W. K.: The Serum Polysaccharide-Protein Ration (PR) as a Measure of Rheumatoid Arthritis Activity, Ann. Int. Med. **41:** 775-779, 1954.
8. Shetlar, M. R., Foster, Janeal V., and Everett, M. R.: Determination of Serum Polysaccharides by the Tryptophane Reaction, Proc. Soc. Exper. Biol. & Med. **67:** 125-130, 1948.
9. Weichselbaum, T. E.: An Accurate and Rapid Method for the Determination of Proteins in Small Amounts of Blood, Serum, and Plasma, Am. J. Clin. Path., Tech. Sect. **10:** 40-49, 1946.
10. Steinbrocker, O., Traeger, C. H., and Batterman, R. C.: Therapeutic Criteria in Rheumatoid Arthritis, J. A. M. A. **140:** 659-662, 1949.
11. Steinbrocker, O., Berkowitz, S., Ehrlich, M., Elkind, M., and Carp, S.: Phenylbutazone Therapy of Arthritis and Other Painful Musculoskeletal Disorders, J. A. M. A. **150:** 1087-1091, 1952.
12. Brodie, B. B., Lowman, E. W., Burns, J. J., Lee, P. R., Chenkin, T., Goldman, A., Weiner, M., and Steele, J. M.: Observations on the Antirheumatic and Physiologic Effect of Phenylbutazone (Butazolidin) and Some Comparisons With Cortisone, Am. J. Med. **16:** 181-190, 1954.
13. Nathan, D. A., Meitus, M. L., Capland, L., and Lev, M.: Death Following Phenylbutazone (Butazolidin) Therapy: Report of a Case, Ann. Int. Med. **39:** 1096-1103, 1953.

# THE EFFECT OF THYROID HORMONE UPON THE DEGRADATION RATE AND MISCIBLE POOL OF RADIOIODINATED HUMAN SERUM ALBUMIN IN MYXEDEMA

ERNEST SCHWARTZ, A.M., M.D.
LOS ANGELES, CALIF.

## INTRODUCTION

EARLY investigators found evidence of negative nitrogen balance in normal dogs which were fed thyroid substance. Rohde and Stockholm[1] cited ten such experiments published between 1895 and 1908. Janney[2] confirmed this effect in the human being. In the adult hypothyroid patient the acute administration of thyroid hormone produces a markedly negative nitrogen balance,[3-6] which has been attributed to the catabolic mobilization of protein-rich myxedema fluid. A similar effect also occurs initially in the cretin.[2, 7, 8] From measurement of the nitrogen, sodium, and potassium released after the injection of thyroxine, Byrom[6] deduced that the myxedematous patient catabolized a depot of extracellular protein, while the euthyroid patient catabolized intracellular protein. On the other hand, thyroid hormone eventually produces a positive nitrogen balance in human cretins[2, 7, 8] and in thyroparathyroidectomized growing rats.[9] It has been found that the biologic half-lives of $I^{131}$-labeled homologous serum albumins in cows, men, dogs, rabbits, and mice are approximately inversely proportionate to the respective metabolic rates of each species.[10] Thyroxine shortened the half-life of labeled albumin in normal rabbits from 5.7 to 4.4 days.[10]

The following studies were designed to determine the degradation rate of $I^{131}$-labeled human albumin and the size of the miscible albumin pool in myxedema before and after thyroid hormone therapy.

## MATERIALS AND METHODS

A. *Subjects.*—The clinical laboratory data for the two patients studied are presented in Table I. In the patient H. N. L. only the miscible albumin pools are reported because the brief duration of one experiment did not permit accurate comparisons of labeled albumin half-lives and degradation rates. Both patients were treated with thyroid hormone for approximately ten weeks before the experiments were repeated. The final dose in each was 2 gr. of desiccated thyroid gland (U.S.P.-Armour) daily, which resulted in the attainment of a clinically euthyroid state, as indicated by loss of edema, greater alertness, and improvement in skin texture, voice, and cold tolerance.

B. *Laboratory Methods.*—A dose of about 300 $\mu$c of iodinated human serum albumin (Abbott) diluted in normal saline solution was injected intravenously into the fasting patient from a weighed syringe. The total number of radioactive counts per second administered was calculated by scintillation-tube counting of an appropriate dilution of an aliquot of the injected solution. The ten-minute plasma specimen was used to calculate approximately the plasma volume. Thereafter, blood specimens were usually drawn daily and centrifuged in tubes containing a trace of heparin powder. Two to five milliliters of plasma was pipetted into a counting tin. If necessary, saline solution was added to make up a final volume of five milliliters. Daily twenty-four-hour urine collections were ob-

From the Radioisotope Unit, Wadsworth Hospital, Veterans Administration Center, and the Department of Medicine, University of California, Los Angeles, Calif.
Received for publication Sept. 11, 1954.

TABLE I. LABORATORY DATA OF TWO PATIENTS WITH MYXEDEMA

| PATIENT | AGE SEX | DIAGNOSIS | DATE OF EXPERIMENT | WGT. (KG.) | B.M.R. (%) | PBI (μG%) | $I^{131}$ UPTAKE | SERUM CHOL. (MG. %) | 24-HR. URINE GONADOTROPIN (M.U.) | 24-HR. URINE 17-K STEROIDS (MG.) |
|---|---|---|---|---|---|---|---|---|---|---|
| J. A. N. GH 37523 | 53 M | Chronic thyroiditis | 7-29-53 (before treatment) | 78.3 | −30 | 4.5, 4.1 | 0 | 367 | < 10 | 5.2 |
| | | | 10-24-53 (after treatment) | 71.0 | + 7 | — | — | 157 | < 10 | 5.0, 6.0 |
| | | | | | | 2.1 (4/54*) | | | 10 (4/54*) | 8.8, 5.5 (5/54*) |
| H. N. L. GH 41641 | 60 M | Idiopathic myxedema | 8-14-53 (before treatment) | 79.2 | −24 | 2.0 | 0 | 700 | < 10 | 5.2, 6.8, 2.9 |
| | | | 11-11-53 (after treatment) | 73.2 | −10 | — | — | 240 | < 10<br>> 10 < 20 (3/54*) | 6.0, 6.0 |

*Dates of later determinations.

tained and 5-ml. aliquots assayed. Samples were assayed by a thallium-activated sodium iodide crystal scintillation tube and autoscaler standardized with a Tracerlab antimony$^{125}$ standard. The tube sensitivity was approximately 2,200 counts per second per microcurie $I^{131}$. Backgrounds varied from 4 to 15 counts per second. In order to keep the counting error below 1 per cent, total counts of 40,960 were often made, using an Eagle preset timer. All counts were corrected by physical decay tables to the time of injection. Total plasma proteins were determined two to three times weekly by the Howe method during the course of the study and were found to be stable. In addition, complete fractionations were done to separate albumin from alpha globulin by the sodium sulfite method.[11] The mean albumin value was used to compute albumin specific activities in terms of counts/sec./mg. albumin. Corrections for radioactivity withdrawn during sampling were made only for the first study with Patient J. A. N., in which many samples were drawn during the first day. In the other studies, this correction was less than 1 per cent and was disregarded. Scintillation tube counting of the neck verified the absence of $I^{131}$ concentration in the area of the thyroid gland. All urines in both patients were protein-free.

Four graphs were plotted as described later from the data for each experiment (Figs. 1 and 2).

*Graph 1. Per cent of administered dose remaining in the body:* The values for per cent dose remaining were calculated by subtracting the cumulative number of counts per second excreted in the urine from the total number of counts per second injected. The curves began with a straight line which was of somewhat steeper slope during the mixing period and which changed to a line of shallower slope. The change in slope was caused by a more rapid rate of urinary excretion during the mixing phase (as seen in Graph 4), presumably related to (1) the initial release of unbound $I^{131}$ (usually not more than 5 per cent of undialyzed preparations[12] and in the experience of this laboratory less than 2 per cent), (2) the breakdown of unusually labile molecules, and (3) possibly the presentation during the mixing period of relatively more labeled albumin to the liver for breakdown, if the liver is the principal site of degradation. The labeled albumin half-life was obtained by extrapolating the second phase of the curve back to the ordinate and determining the time on the curve corresponding to half the ordinate intercept.

*Graph 2. Albumin specific activity:* The initial phase of this graph is related to the mixing process. Extrapolation of the straight-line component to the ordinate permits the calculation of half-life in the same fashion as with Graph 1. The albumin specific activity corresponding to the ordinate intercept of this extrapolated line can be divided into the total number of counts injected to obtain a value for the miscible albumin pool, since the

value of the intercept theoretically corresponds to that value of specific activity which would be obtained if equilibrium with the total pool were established immediately after injection. However, the miscible albumin pool may be too large when calculated in this way because the total number of counts per second injected often contains a certain proportion more rapidly excreted in the urine during the mixing phase. On the other hand, the value of miscible pool may be too small if the curve is prematurely extrapolated before the degradation rate of the slowest component is measured.[12] The ordinate intercept would then be too high. For these reasons, the values of albumin miscible pool derived from Graph 2 were considered unreliable, and the results obtained from direct calculation of isotope dilution (Graph 3) were utilized.

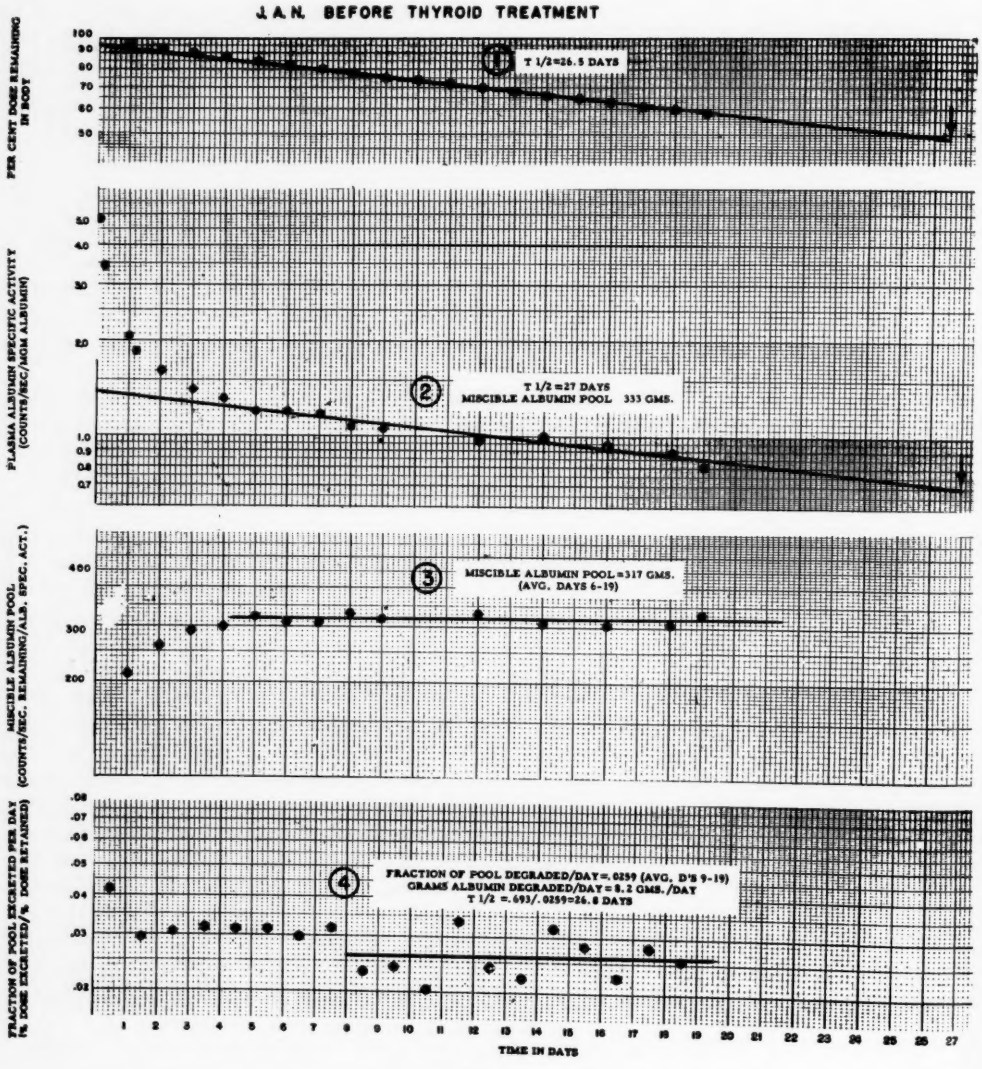

J.A.N. BEFORE THYROID TREATMENT
PER CENT DOSE REMAINING IN BODY
T 1/2=26.5 DAYS
PLASMA ALBUMIN SPECIFIC ACTIVITY (COUNTS/SEC./MGM ALBUMIN)
T 1/2 =27 DAYS
MISCIBLE ALBUMIN POOL 333 GMS.
MISCIBLE ALBUMIN POOL (COUNTS/SEC. REMAINING/ALB. SPEC. ACT.)
MISCIBLE ALBUMIN POOL=317 GMS.
(AVG. DAYS 6-19)
FRACTION OF POOL SECRETED PER DAY IN DOSE SECRETED/% DOSE RETAINED)
FRACTION OF POOL DEGRADED/DAY=.0259 (AVG. D'S 9-19)
GRAMS ALBUMIN DEGRADED/DAY=8.2 GMS./DAY
T 1/2 =.693/.0259=26.8 DAYS
TIME IN DAYS

Fig. 1.

*Graph 3. Albumin miscible pool:* This curve is obtained from the quotient of the actual number of counts per second remaining in the body at any time, divided by albumin specific activity—or counts/sec./mg. albumin. It is related to the reciprocal of

the distribution curve plotted by Berson and associates.[12] The quotient represented by the straight portion of this curve should be an accurate representation of the miscible pool at equilibrium, since it has been mathematically demonstrated that iodide is for all practical purposes excreted immediately after its release from degraded albumin.[12] The

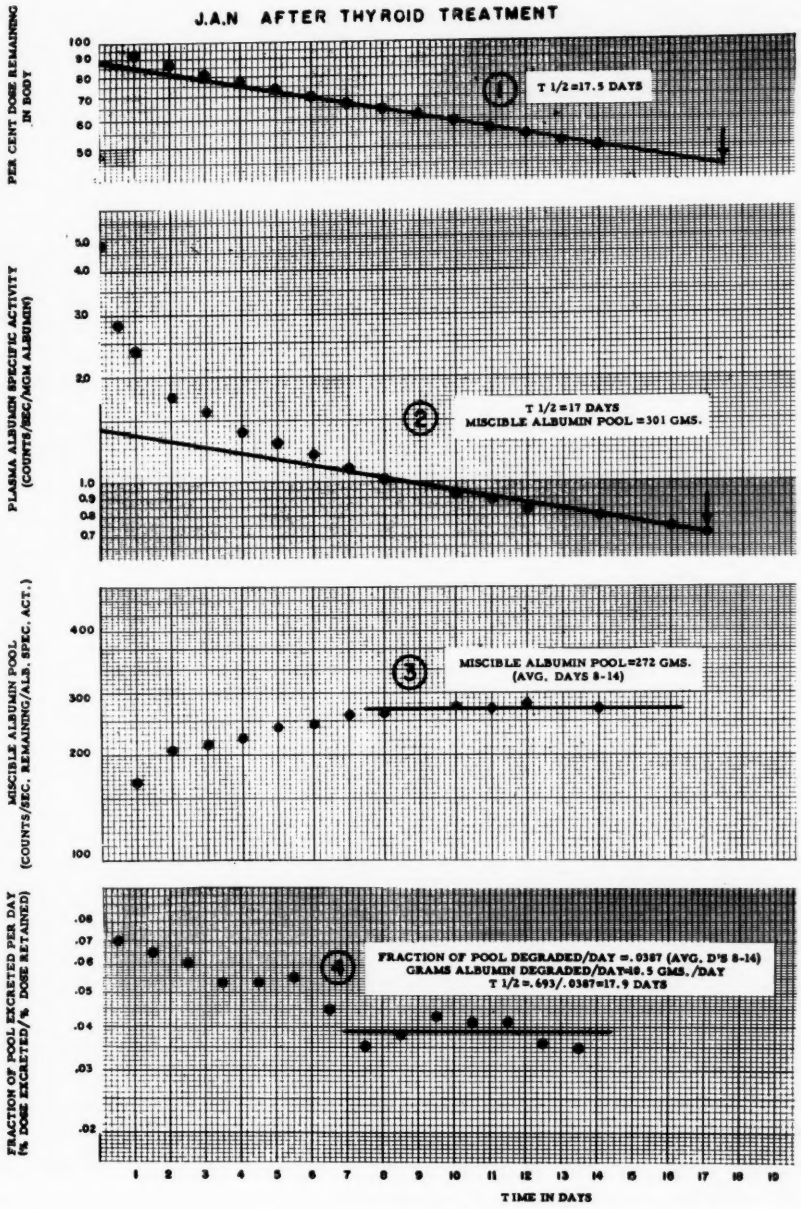



Fig. 2.

approach of this curve to a horizontal asymptote at equilibrium demonstrates that inorganic $I^{131}$ is not sequestered anywhere in the body or lost through incomplete urine collections. If either of these events occurred, the calculated pool value would grow steadily larger.

*Graph 4. Fraction of miscible albumin pool excreted per day:* This fraction is given by the quotient of the per cent of initial dose excreted each day divided by the average per cent of dose remaining during that day (or number of counts per second excreted daily divided by average number of counts per second retained). Initially, the fraction excreted is comparatively larger, probably because of the factors outlined previously. After several days the values began to oscillate at a lower level. These lower values of the fraction were averaged. The actual number of grams of albumin degraded per day could be estimated by multiplying the fraction by the size of the pool as determined from Graph 3. The half-life could be readily estimated from the formula:

$T_{½}$ = 0.693 divided by the fraction degraded daily.

## RESULTS

The initial half-life of about 27 days was reduced to 17 days after 10 weeks of treatment with thyroid extract (Table II). The calculations of half-life by the various methods were in close agreement. The mean value for miscible albumin pool (Graph 3) was reduced from 317 to 272 Gm., a net loss of 45 Gm. Turnover rate rose from 2.6 to 3.9 per cent of the pool degraded daily, or from 8.2 Gm. to 10.5 Gm. of albumin degraded daily, before and after thyroid therapy. This was a net increase of 28 per cent in grams of albumin degraded daily. From the values of plasma volume, plasma albumin levels, and miscible albumin pool, the extravascular albumin pools could be calculated (Table III). These were 222 Gm. and 182 Gm. before and after thyroid therapy, respectively—a net decrease of 40 Gm. or 18 per cent. Total intravascular albumin did not change appreciably.

TABLE II. J. A. N. CALCULATION OF $T_{½}$, ALBUMIN MISCIBLE POOL, AND ALBUMIN DEGRADATION RATE BEFORE AND AFTER THYROID THERAPY

| | GRAPH 1 % DOSE RETAINED | | GRAPH 2 ALB. SPEC. ACT. | | GRAPH 3 ALB. MISC. POOL | | GRAPH 4 FRACT. POOL PER DAY | |
|---|---|---|---|---|---|---|---|---|
| | BEFORE | AFTER | BEFORE | AFTER | BEFORE | AFTER | BEFORE | AFTER |
| $T_{½}$ (days) | 26.5 | 17.5 | 27 | 17 | | | 26.8† | 17.9† |
| Albumin misc. pool | | | 333 g. | 301 g. | 317 g.* | 272 g.* | | |
| Fraction of pool degraded per day | | | | | | | 2.59% | 3.87% |
| Gm. albumin degraded per day | | | | | | | 8.2 | 10.5 |

*These values of the pool were used for calculation of albumin degradation in grams per day.

†These values of half-life were calculated from fraction of pool degraded per day.

In a second case of myxedema, although accurate comparisons of half-lives and degradation rates were not possible, the values of miscible albumin pools became stable sufficiently early to permit accurate estimations (Table IV). In this patient, the total albumin pool decreased from 326 to 239 Gm., a net loss of 87 Gm. Extravascular albumin decreased from 236 to 148 Gm., a net loss of 88 Gm. or 37 per cent. The increase in plasma volume (7 per cent) was probably not significant.

*Discussion.*—The validity of these figures for half-lives may be readily estimated by comparing the values of miscible albumin pool determined from extrapolation of the albumin specific activity curve (Graph 2), and from Graph 3, which represents the pool directly. Before thyroid therapy the pool

value from Graph 2 was 5 per cent greater, and after thyroid therapy 11 per cent greater than the pool value from Graph 3. This percentile comparison is a direct measure of the homogeneity of the $I^{131}$-labeled albumin for the most slowly degraded component,[12] and also is a check on the accuracy of the extrapolation in Graph 2. If the curve of Graph 2 were prematurely extrapolated before the degradation rate of the slowest component could be accurately determined, the albumin pool of Graph 2 would be smaller than the pool of Graph 3, instead of vice versa. The half-lives as determined by Graphs 1 and 4 are in excellent agreement with the values of Graph 2. It, therefore, seems clear that the degradation rate of $I^{131}$-labeled albumin (and, by the definition of equilibrium, the synthesis rate of endogenous albumin) is grossly decreased in myxedema, and that the half-life is correspondingly prolonged, compared with findings in the euthyroid state in the same individual.

TABLE III. J. A. N. TOTAL PLASMA AND EXTRAVASCULAR ALBUMIN

| TIME OF STUDY AND RISA* LOT NO. | PLASMA VOLUME (ML.) | PLASMA ALBUMIN (G.%) | TOTAL PL. ALBUMIN (G.) | ALBUMIN MISC. POOL (G.) | EXTRA-VASCULAR ALBUMIN (G.) | EXTRA-VASCULAR ALBUMIN LOST (G.) |
|---|---|---|---|---|---|---|
| Before thyroid (Lot 714-089-5) | 2720 | 3.5 | 95 | 317 | 222 | 40 |
| After thyroid (Lot 714-175-8) | 2810 | 3.2 | 90 | 272 | 182 | |

*$I^{131}$ albumin, Abbott.

TABLE IV. H. N. L. TOTAL PLASMA AND EXTRAVASCULAR ALBUMIN

| TIME OF STUDY AND RISA* LOT NO. | LENGTH OF EXPT. (DAYS) | PLASMA VOLUME (ML.) | PLASMA ALBUMIN (G.%) | TOTAL PL. ALBUMIN (G.) | ALBUMIN MISC. POOL (G.) | EXTRA-VASCULAR ALBUMIN (G.) | EXTRA-VASCULAR ALBUMIN LOST (G.) |
|---|---|---|---|---|---|---|---|
| Before thyroid (Lot 714-091-11) | 11 | 2740 | 3.3 | 90 | 326 | 236 | 88 |
| After thyroid (Lot 714-211-40) | 13 | 2930 | 3.1 | 91 | 239 | 148 | |

*$I^{131}$ albumin, Abbott.

When mixing is complete, the Graph 3 miscible albumin pool values remain relatively constant, regardless of the length of experiment or of varying rates of degradation, provided that the released iodide is rapidly excreted and that urinary collections are complete. Thus, it is evident that the state of myxedema is associated with an increase in the miscible albumin pool, which is almost entirely due to an increase in extravascular albumin. Patient J. A. N. lost 40 Gm. of extravascular albumin, or 18 per cent of his extravascular pool, during thyroid therapy. Patient H. N. L. lost 88 Gm. of extravascular albumin, or 37 per cent of his extravascular pool. These figures may be compared with Byrom's rough estimate of the mobilizable extravascular protein depot in myxedema as 100 to 200 Gm.[6]

Our values for albumin pool and half-life in these patients after thyroid therapy do not entirely agree with those of other investigators. $I^{131}$-labeled albumin was calculated by Sterling[13] to have an average half-life in normal

human beings of 10.5 ± 1.5 days and a miscible pool of 259 ± 40 Gm. These values were obtained from extrapolation of albumin specific activity curves, which may be unreliable because premature extrapolation results in steeper slopes, shorter half-lives, and smaller miscible pools. The data of Berson and associates[12] from eleven noncirrhotic patients tested with lots of iodinated albumin free from rapidly degraded components reveal an average half-life of 17.1 ± 3.4 days and an average total exchangeable albumin of 327 ± 63 Gm. (our calculations for ± 2 standard deviations). This figure for miscible pool is considerably larger than the values of 272 and 239 Gm. herein reported for the two subjects in the euthyroid state. It may be that the Berson group's determinations of plasma albumin were falsely high because of the inclusion of alpha globulin in the conventional Howe sodium sulfate fractionations. Alpha globulin levels in our patients were approximately 1.7 Gm. per cent, and their albumin levels by the Howe method were in the range of 5 Gm. per cent. High values of albumin would produce erroneously low albumin specific activities and correspondingly high values for the pool although the calculation of half-life would not be affected. The figures of Berson's group for $I^{131}$-labeled albumin half-life in noncirrhotic persons are in general accord with the figure of Dixon's group of 15 days for 11 normal human males[10] and with data obtained using albumin labeled by other isotopes.[14, 15] It would therefore seem that the half-life of $I^{131}$-labeled albumin is a reasonable approximation of the half-life of endogenously synthesized albumin.

Since the two patients herein studied appeared to have pituitary and adrenal hypofunction both before and after treatment with thyroid hormone, it would appear that thyroid hormone had an independent effect upon their metabolism of labeled albumin, whereas Hoberman and Graff's experiments[16] in growing thyroparathyroidectomized rats suggested a thyroid-pituitary relationship in protein metabolism. Determinations in both patients several months later revealed a slow return of gonadotropin levels toward normal, thus excluding primary pituitary disease. However, the levels of urinary 17-ketosteroid and gonadotropin excretion are only partial indices of over-all pituitary and adrenal cortical hormonal production. Variations in the output of other pituitary and adrenal hormones were not excluded.

The studies herein described do not illuminate the mechanism producing an increased extravascular albumin pool in myxedema. This may be partially related to changes in capillary permeability. However, the rapid mobilization of fluid and nitrogen by the acute administration of thyroid hormone to the myxedematous patient seems to indicate that the enlarged pool is in some way a consequence of altered protein metabolism.

## SUMMARY

The half-life of $I^{131}$-labeled human serum albumin in a patient with myxedema appeared to be considerably prolonged, and the degradation rate correspondingly slowed. The attainment of a clinically euthyroid state was associated with a return of the biologic half-life and degradation rate toward normal.

The extravascular miscible albumin pool in two myxedematous patients was reduced by 18 and 37 per cent, respectively, after ten weeks of treatment with thyroid extract. Plasma volume and total plasma albumin were not significantly changed.

The author is indebted to Mr. Max Fields for technical assistance. He is grateful to Dr. Franz K. Bauer and Dr. William H. Blahd for much helpful advice, and to Dr. Samuel H. Bassett and Dr. David H. Solomon for constructive criticism of the manuscript.

REFERENCES

1. Rohde, A., and Stockholm, M.: The Increase in Nitrogen Metabolism of the Dog Following the Administration of Desiccated Thyroid Gland, J. Biol. Chem. **37:** 305-316, 1919.
2. Janney, N. W.: III. Studies in Thyroid Therapy: The Effects of Thyroid Hormone as Determined by a Clinical, Metabolic and Dietetic Investigation. New Points of View on Thyroid Function in Health and Disease, Arch. Int. Med. **22:** 187-216, 1918.
3. Ord, W. M., and White, E.: Clinical Remarks on Certain Changes Observed in the Urine in Myxedema After the Administration of Glycerine Extract of Thyroid Gland, Brit. M. J. **2:** 217, 1893.
4. Boothby, W. M., Sandiford, I., Sandiford, K., and Slosse, J.: The Effect of Thyroxine on the Respiratory and Nitrogenous Metabolism of Normal and Myxedematous Subjects. I. A Method Studying the Reserve or Deposit Protein With a Preliminary Report of the Results Obtained, Ergebn. Physiol. **24:** 728-756, 1925.
5. Albright, F., Bauer, W., and Aub, J. C.: Studies of Calcium and Phosphorus Metabolism. VIII. The Influence of the Thyroid Gland and the Parathyroid Hormone Upon the Total Acid Base Balance, J. Clin. Invest. **10:** 187-219, 1931.
6. Byrom, F. B.: The Nature of Myxedema, Clin. Sc. **1:** 273-285, 1934.
7. Johnston, J. A., and Maroney, J. W.: Factors Affecting Retention of Nitrogen and Calcium in the Period of Growth. I. Effect of Thyroid on Nitrogen Retention, Am. J. Dis. Child. **58:** 965-982, 1939.
8. Wilkins, J.: Effects of Thyroid and of Androgens on the Nitrogen and Fluid Balances of a Cretin, Conference on Metabolic Aspects of Convalescence **9:** 158-167, 1945.
9. Rupp, J., Paschkis, K. E., and Cantarow, A.: Influence of Thyroxine on Protein Metabolism, Endocrinology **44:** 449, 1949.
10. Dixon, F. J., Maurer, P. M., and Deichmiller, M. P.: Half-Lives of Homologous Serum Albumins in Several Species, Proc. Soc. Exper. Biol. & Med. **83:** 287-288, 1953.
11. Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F.: Studies in Serum Proteins: V. A Rapid Procedure for the Estimation of Total Protein, True Albumin, Total Globulin, Alpha Globulin, Beta Globulin and Gamma Globulin in 1.0 ml. of Serum, Am. J. Clin. Path. **18:** 723-730, 1948.
12. Berson, S. A., Yalow, R. S., Schreiber, S. S., and Post, J.: Tracer Experiments With $I^{131}$ Labeled Human Serum Albumin: Distribution and Degradation Studies, J. Clin. Invest. **32:** 746-768, 1953.
13. Sterling, K.: The Turnover Rate of Serum Albumin in Man as Measured by $I^{131}$ Tagged Albumin, J. Clin. Invest. **30:** 1228-1237, 1951.
14. London, I. M.: Studies on the Rates of Formation of Serum Proteins in Man. The Robert Gould Research Foundation, Inc., Cincinnati, Symposia on Nutrition **2:** 72-82, 1950.
15. Volwiler, W., Fremont-Smith, K., and MacMartin, M. P.: Turnover Rates of Serum Proteins in Cirrhotic Man, J. Clin. Invest. **31:** 668, 1952.
16. Hoberman, H. D., and Graff, J.: Influence of Thyroxine on the Metabolism of Amino Acids and Proteins During Fasting, Yale J. Biol. & Med. **23:** 195-198, 1950.

# LACK OF EFFECT OF FLUORINE INGESTION ON UPTAKE OF IODINE$^{131}$ BY THE THYROID GLAND

J. Elliot Levi, M.D., and Hannah E. Silberstein, M.S.
Baltimore, Md.

## INTRODUCTION

BECAUSE of the close chemical association between fluorine and iodine, there has been considerable interest for many years in the metabolic interrelationships of these two halides and their effect on the thyroid gland.

In 1926 Goldemberg[2] revived the suggestion of Maumené made in 1854[1] that toxic amounts of fluoride might be the cause of endemic goiter. Goldemberg[3] further reported a therapeutic effect when large amounts of fluoride were administered to patients with hyperthyroidism.

Gorlitzer[4] in 1932 and again in 1950 confirmed this effect, but stated that patients could not tolerate fluorides in the large quantities required by mouth, because of gastric disturbances. He and others attempted parenteral administration of this compound with apparently good results, but this form of therapy has not been pursued.

In 1941 Wilson[5] observed a high degree of dental fluorosis (mottled teeth) in an area of the Punjab, India, in which endemic goiter was coexistent, and also recorded a similar association in Somerset, England.

In 1948 Steyn[6] found a relationship between the incidence of endemic goiter and the occurrence of excessive quantities of fluoride in the drinking water, in spite of adequate iodine supply, in some regions of the Cape Province.

On the other hand, Wilson[5] attributed the absence of goiter in the community of Maldon, Essex, where the drinking water contains 5 ppm of fluoride, to its unusually high content of iodine, and to the fact that much fish is eaten by the people in that area. Spira[7] recently recalled that in 1942 he had found no coincidence of mottled teeth and struma in Great Britain. However, the people examined were mainly young men and women serving in the army and the finding of a struma prior to induction would have been reason for rejection from military service. Spira reviewed the pathologic findings quite consistently observed in the thyroids of experimental animals in his own study and in those of Goldemberg,[2] Cristiani,[8] and others. On levels of chronic fluoride ingestion that were sooner or later lethal, they saw general cellular hyperplasia, sometimes dilated vesicles poor in colloid, parenchymatous proliferation, and occasionally interstitial cell hyperplasia. Chaneles,[9] however, in rats on somewhat smaller doses of fluoride, found none of these changes in thyroid tissue. Spira, therefore, pointed out the need for further investigation before drawing conclusions from these observations in respect to the human thyroid gland.

On Nov. 26, 1952, fluoridation of the water supply for Baltimore was instituted in order to reduce the incidence of dental decay.[10] The fluoride ion content was soon stabilized at 1 ppm. It seemed desirable to determine what effect, if any, this rate of fluoride ingestion would have on the uptake of radioactive iodine by the thyroid glands of euthyroid subjects. Accordingly, the following study was undertaken.

From the Departments of Medicine and Radiology and the Radioisotope Laboratory of Sinai Hospital, Baltimore, Md.

Received for publication Sept. 18, 1954.

## METHOD

Thirty volunteer patients at the Deershead State Hospital in Salisbury, Maryland, were selected for this study. Although they were suffering from a variety of chronic diseases, none of them had any history or clinical signs of thyroid deviation. None of these subjects were exposed to fluoridated water (natural or artificial) for a period of at least six months prior to or during the ten-week study period. The natural fluoride content of Salisbury city water is less than 0.5 ppm, although several communities in the general area have a natural fluoride content greater than 1 ppm.

Radioactive iodine-uptake studies consisted of the administration of 50 $\mu$c of $I^{131}$ by mouth and a twenty-four-hour uptake measurement over the neck, using a 12 cm. skin-tube distance and a conventional Geiger-Müller counter. All cervical readings were corrected for background by readings taken over the lower thigh. From the day following the control tracer tests, the subjects were given 4 mg. of fluoride ion per day as sodium fluosilicate in a single daily dose of 2 ounces of distilled water for the first three weeks and in two daily divided doses of 2 mg. each for the remaining seven weeks. The material was in the same chemical form as that used to fluoridate the Baltimore city water supply and the amount was estimated to be slightly in excess of the average daily fluoride intake of normal subjects in Baltimore. The daily dose would have been equal to that ingested in 4 L. of fluoridated water containing 1 ppm of fluoride ion. At the end of the first three weeks a second tracer test was performed on all but one of the subjects. A small correction for residual count from the first test had to be applied to the readings at this time. Seventeen of the group remained on the 4 mg. per day fluoride regimen for an additional seven weeks. Repeat radioiodine uptake studies were performed on this group after ten weeks of continuous fluoride ingestion.

## RESULTS

In the first, or control, test the corrected twenty-four-hour uptake readings ranged from 1.8 to 9.02 counts per second representing, according to our standard reference, from 10 to 51 per cent of the dose of $I^{131}$ in the thyroid gland. All but three of these fell within our euthyroid range with counts of 2.1 to 7.4, equivalent to 12 to 42 per cent of dosage. Attention should be called to these three exceptions. The only suggestively elevated count, 9.02 or 51 per cent, was seen in E. F., whose reading had to be taken at the bedside under difficult positioning conditions, and whose later tests were found to fall in the euthyroid range. Two of the subjects showed uptakes of 10.0 and 10.5 per cent of the dose, which is at the lower limit of normal. C. Wr. was on 12 to 14 tablets of Prostigmine methyl bromide daily for control of myasthenia gravis, and the bromine content might have accounted for some suppression of iodine uptake. C. Le., a patient with bronchiectasis, had had a Lipiodol instillation two years prior to this test, and the prolonged effect of such iodine compounds is well known.

At the time of the second tracer test, after three weeks of the experimental fluoride regimen, no significant changes in uptake at twenty-four hours could be seen. The readings for the whole group, including the three exceptions mentioned, ranged from 2.0 to 7.6 counts per second over the gland. In comparison with the control findings, some were increased a little and a nearly equal number were decreased. The over-all difference between the two sets of determinations was 1.92 counts per second higher in the second study. The average difference was only 0.07. Therefore, administration of fluoride was continued for an additional seven weeks to seventeen members of the original group.

TABLE I. TWENTY-FOUR-HOUR UPTAKE OF $I^{131}$ IN THYROID GLANDS OF SUBJECTS BEFORE AND AFTER A TEN-WEEK COURSE OF SODIUM FLUOSILICATE

| SUBJECT | COUNTS/SECOND/50 μC DOSE | | DIFFERENCE IN COUNTS/SECOND |
|---|---|---|---|
| | CONTROL | EXPERIMENTAL | |
| M. H. | 5.54 | 0.90 | −4.64 |
| E. F. | 9.02 | 5.49 | −3.53 |
| C. Le. | 1.84 | 0.20 | −1.64 |
| C. S. | 6.41 | 5.56 | −0.85 |
| D. D. | 4.47 | 3.71 | −0.76 |
| C. Wi. | 4.81 | 4.52 | −0.29 |
| E. A. | 4.16 | 4.20 | 0.04 |
| H. F. | 3.56 | 3.74 | 0.18 |
| C. La. | 4.85 | 5.10 | 0.25 |
| M. B. | 3.14 | 3.74 | 0.60 |
| S. B. | 3.65 | 4.28 | 0.63 |
| K. D. | 4.78 | 5.41 | 0.63 |
| L. H. | 2.80 | 3.45 | 0.65 |
| C. Wr. | 1.78 | 2.92 | 1.14 |
| J. B. | 3.54 | 4.81 | 1.27 |
| F. T. | 3.56 | 5.49 | 1.93 |
| G. J. | 3.04 | 4.97 | 1.93 |
| Sum of differences between 2 tests: | | | −2.47 |
| Average difference between 2 tests: | | | −0.15 |

As seen in Table I, there is a good deal of variability between the first and third studies, but the total difference between the two series amounts to −2.47 counts per second with an average difference of −0.15 count per second. This cannot be considered significant especially as in ten of the cases there was no essential change. Two of the third series of uptakes fell below the euthyroid range. That of C. Le. can be accounted for, as we have already pointed out, on the basis of residual iodide from the material used for bronchograms two years before. The low uptake in the case of M. H., however, is unexplained.

## SUMMARY AND CONCLUSION

There are historical reasons to suspect that there may be a competitive relationship between fluorine and iodine in the metabolism of the thyroid gland. The iodide-trapping function of the thyroid as measured by radioiodine studies has proved to be an exceedingly sensitive test of one thyroid function and of the relationship of interfering substances such as thiocyanate. However, in seventeen euthyroid subjects, no effect on iodide trapping was demonstrated by the daily administration of 4 mg. of fluoride ion in the form of sodium fluosilicate over a ten-week period. Therefore, a dilute solution of fluoride in drinking water of the order of 1 ppm has no effect on radioiodine uptake studies of thyroid function.

The authors wish to express their gratitude to Dr. Abel Wolman of The Johns Hopkins University, Drs. Robert H. Riley, James McCallum, and Vernor Juerman of the Maryland State Health Department, and H. Berton McCauley of the Baltimore City Health Department, whose kind help and generous cooperation made this study possible.

## REFERENCES

1. Maumené, E.: Expériences pour déterminer l'action des fluorures sur l'économie animale, Compt. rend. Acad. sc. **39**: 538, 1854.
2. Goldemberg, L.: Action physiologique des fluorures, Compt. rend. Soc. biol. **95**: 1169, 1926.

3. Goldemberg, L.: Traitement de la maladie de Basedow et de l'hyperthyroidisine par le fluor, Presse méd. **38:** 1751, 1930.
4. Gorlitzer, V.: Ein neuer Weg zur Behandlung der Thyreotoxikose mit Fluorwasserstoffsäure, Med. Klin. **28:** 717, 1932; Personal communication to L. Spira, 1950.
5. Wilson, D. C.: Fluorine in the Aetiology of Endemic Goitre, Lancet **1:** 211, 1941.
6. Steyn, D. G.: Fluorine and Endemic Goitre, South African M. J. **22:** 525, 1948.
7. Spira, L.: Further Study of the Role Played by Fluorine in the Causation of Disease, Exper. Med. & Surg. **10:** 63, 1952.
8. Cristiani, H.: Altérations de la glande thyroïde dans l'intoxication fluorée, Compt. rend. Soc. biol. **103:** 554, 1930.
9. Chaneles, J.: Action de l'iode sur la fluorose chronique, Compt. rend. Soc. biol. **102:** 863, 1929.
10. Announcement, Baltimore Health News **30:** 81, 1953.

# THE USE OF THE URINARY PIGMENT : CREATININE RATIO AS A MEASURE OF BASAL METABOLIC RATE AND THYROID ACTIVITY

FERRIN B. MORELAND, PH.D., AND ARTHUR E. GURGIOLO, M.A.
HOUSTON, TEX.

DRABKIN[2] in 1927 reported that the amount of normal pigment excreted in the urine is proportional to the basal metabolic rate. Proceeding from this finding, Ostow and Philo[4] made a series of comparisons between pigment excretion and basal metabolic rate (B.M.R.) as determined by oxygen consumption. They found good correlation between the rate of the excretion of urinary pigment and the oxygen B.M.R. However, to eliminate the necessity of measuring urinary volumes per unit time and because, in the presence of thyroid dysfunction, the excretion of pigment and of creatinine are expected to vary in opposite directions thus giving wider excursions than either substance alone, Ostow and Philo chose to relate the pigment output to the amount of creatinine excreted at the same time.

Vorzimer and co-workers[3, 5, 6] continued this work, estimating B.M.R. from the urinary pigment : creatinine (P/C) ratio in a considerable number of cases and comparing it with oxygen B.M.R. and with the clinical status of the patient.

The present study involves a clarification of the method of determining the urinary pigment : creatinine ratio, which has never been completely and correctly published as it has been used. The basal metabolic rate derived from this ratio is compared with the basal metabolic rate as determined by oxygen consumption, with the clinical status of the patient, and with the radioiodine uptake.

## METHODS

The amount of the normal urinary pigment cannot be determined in the presence of bilirubin, hemoglobin, or other extraneous pigments such as result from the ingestion of beets, rhubarb, large quantities of riboflavin, or certain dyes. A valid P/C ratio cannot be obtained in patients who have renal disease with nitrogen retention, in patients with severe liver damage, and in some patients with chronic heart disease.

*Specimen.*—The determinations should be made on a morning sample of urine collected after the night urine has been voided. To prevent variations in the apparent concentration of urinary pigment due to chemical alterations after voiding, the sample of urine should be refrigerated immediately. The pigment reading should be made as soon as possible, and not later than two hours after voiding.

*Pigment.*—Pigment determination is made after the urine sample is centrifuged at high speed for ten minutes to remove any sediment. A portion of the supernatant urine is then (a) placed in a 10 by 75 mm. cuvette and its absorbance (optical density) is determined at a wave length of 420 mμ with a Coleman Junior spectrophotometer or (b) placed in a standard Klett tube and is read in a Klett-Summerson photoelectric colorimeter using a No. 42 blue filter.

From the Department of Biochemistry, Baylor University College of Medicine, and the Veterans Administration Hospital, Houston, Tex.
Received for publication Sept. 11, 1954.

*Creatinine.*—Creatinine is determined by some modification of the Jaffe reaction such as the following: The urine is diluted fivefold with distilled water and mixed. Into one 10 by 75 mm. cuvette is measured 0.1 ml. of the diluted urine; into a second, 0.1 ml. of water for a blank. To each tube 2 ml. of saturated picric acid and 0.15 ml. of 10 per cent sodium hydroxide are added. The tubes are mixed, let stand for ten minutes, and read at 520 mμ in the Coleman, set to zero absorbance with the blank. The creatinine concentration of the urine is determined from a graph made by plotting the readings obtained using suitable creatinine standards in place of the urine. The creatinine concentration is expressed in milligrams of creatinine per milliliter of undiluted urine. If it is desired to use a Klett-Summerson photoelectric colorimeter instead of the Coleman for this determination, a volume of each of the three solutions double those given previously is measured into a Klett tube and read at 10 minutes with the No. 54 green filter.

*Basal Metabolic Rate.*—B.M.R. is then calculated from one of the following formulas[6]:

Coleman:

For males, B.M.R. $= 57.0 + 217\ A/C$

For females, B.M.R. $= 54.7 + 191\ A/C$

Klett:

For males, B.M.R. $= 57.0 + 0.25\ P/C$

For females, B.M.R. $= 54.7 + 0.22\ P/C$

where $A$ is the absorbance as read in the Coleman, $P$ is the Klett reading, and $C$ is the creatinine concentration in milligrams per milliliter of urine, and B.M.R. is in per cent of normal (in the following discussion, 100 is subtracted from these values to give per cent deviation from normal).

## RESULTS

Forty-six determinations of P/C ratio were made on twenty-two adult male patients on the same morning as the measurement of B.M.R. by oxygen consumption; an evaluation of the clinical status of the patient was also obtained. The P/C ratios ranged from 87 to 523 (mean 229, standard deviation 86), the B.M.R.'s calculated from the P/C ratio varied from –22 to +89 per cent (mean +14 per cent, s = 21), and the oxygen B.M.R.'s were from –30 to +65 per cent (mean +13 per cent, s = 22). The coefficient of correlation between the two tests was much lower than that of Vorzimer's group, but a little better than found by Ostow and Philo (Table I).

TABLE I. COEFFICIENT OF CORRELATION BETWEEN P/C AND OXYGEN B.M.R.

| | MALES | FEMALES |
|---|---|---|
| Ostow and Philo | 0.30 | 0.75 |
| Vorzimer, Cohen, and Joskow | 0.88 | 0.92 |
| Present study | 0.36 | |

In order to compare the tests qualitatively, the results were grouped as low, normal, or high. By the use of +11 per cent as the upper limit of normal, we obtained slightly better agreement between the tests than with the use of +15 per cent. On this basis, the two tests agreed in 61 per cent of the observations and disagreed in 39 per cent, with P/C ratio being high in 15 per cent and low in 24 per cent (Table II).

The choice of +11 per cent as the upper limit of normal for the B.M.R. arose from a comparison of the test results with the clinical status of the patients (Table III). The data indicate that the B.M.R., especially as derived from the

P/C ratio, is a more valid diagnostic criterion when these narrower limits of normal are used and that the P/C ratio is less reliable than the oxygen consumption.

TABLE II. COMPARISON BETWEEN B.M.R. BY P/C AND BY $O_2$

| | PRESENT STUDY | | VORZIMER, COHEN, AND JOSKOW | OSTOW AND PHILO |
|---|---|---|---|---|
| RANGE OF NORMAL | −10 TO +15 | −10 TO +11 | −10 TO +15 | −10 TO +15 |
| *Both tests agreeing* | | | | |
| Both high | 10 | 15 | 51 | 16 |
| Both normal | 14 | 12 | 108 | 17 |
| Both low | 1 | 1 | 8 | 1 |
| Total | 25 | 28 | 167 | 34 |
| *P/C lower than $O_2$* | | | | |
| $O_2$ high, P/C normal | 8 | 4 | 8 | 15 |
| $O_2$ high, P/C low | 1 | 1 | 0 | 0 |
| $O_2$ normal, P/C low | 2 | 2 | 7 | 2 |
| Total | 11 | 7 | 15 | 17 |
| *P/C higher than $O_2$* | | | | |
| $O_2$ low, P/C high | 0 | 0 | 0 | 0 |
| $O_2$ low, P/C normal | 5 | 5 | 17 | 8 |
| $O_2$ normal, P/C high | 5 | 6 | 9 | 4 |
| Total | 10 | 11 | 26 | 12 |

TABLE III. COMPARISON OF THE TWO B.M.R.'S WITH CLINICAL STATUS

| | RANGE OF NORMAL | | | |
|---|---|---|---|---|
| | −10 TO +15 | | −10 TO +11 | |
| | P/C | $O_2$ | P/C | $O_2$ |
| *B.M.R. corresponds with status* | | | | |
| Hyperthyroid, B.M.R. high | 10 | 15 | 15 | 16 |
| Euthyroid, B.M.R. normal | 15 | 17 | 14 | 17 |
| Hypothyroid, B.M.R. low | 0 | 3 | 0 | 3 |
| Total correct tests | 25 | 35 | 29 | 36 |
| *B.M.R. below status* | | | | |
| Hyperthyroid, B.M.R. normal | 8 | 3 | 3 | 2 |
| Hyperthyroid, B.M.R. low | 0 | 0 | 0 | 0 |
| Euthyroid, B.M.R. low | 4 | 3 | 4 | 3 |
| Total low tests | 12 | 6 | 7 | 5 |
| *B.M.R. above status* | | | | |
| Hypothyroid, B.M.R. high | 0 | 0 | 0 | 0 |
| Hypothyroid, B.M.R. normal | 4 | 1 | 4 | 1 |
| Euthyroid, B.M.R. high | 5 | 4 | 6 | 4 |
| Total high tests | 9 | 5 | 10 | 5 |

A comparison of the two B.M.R.'s with each other and with the clinical status is summarized in Table IV. In this table, only forty-one observations are recorded in order to compare our results with those of Vorzimer's group, who found that the P/C ratio is an unreliable guide to basal metabolism in patients with hypothyroidism. For this reason our five observations on such patients were not included in Table IV. Our data indicate that when the P/C and oxygen B.M.R.'s do not agree, the oxygen value is the more likely to be correct.

TABLE IV. COMPARISON AMONG B.M.R.'S AND THE CLINICAL STATUS

| | VORZIMER AND COHEN | FRIEDMAN, MARCUS, AND VORZIMER | PRESENT STUDY |
|---|---|---|---|
| | *(per cent of observations)* | | |
| P/C, $O_2$, and clinical status all correspond | 86 | 61 | 61 |
| P/C, but not $O_2$, corresponds with clinical status | 10 | 29 | 10 |
| $O_2$, but not P/C, corresponds with clinical status | 4 | 7 | 20 |
| P/C and $O_2$ correspond, but not with clinical status | | 3 | 7 |
| No two correspond | | | 2 |
| Total observations | 593 | 273 | 46 |

TABLE V. COMPARISON OF P/C B.M.R. WITH $I^{131}$ UPTAKE

| | |
|---|---|
| P/C corresponds with uptake | 8 |
| P/C below uptake | 5 |
| P/C above uptake | 2 |
| Total | 15 |

The P/C ratios were also determined on fifteen patients on whom radioiodine uptake studies were made. The results, recorded in Table V, are in accord with those discussed previously. It can be seen that the agreement of the P/C B.M.R. with $I^{131}$ uptake is almost exactly the same as the agreement of the P/C test with the oxygen test shown in the first column of Table II or as the agreement of the P/C test with the clinical status as seen in the first column of Table III.

## DISCUSSION

The method for determining the P/C ratio was not explicitly stated by previous investigators. Both groups apparently used the Klett reading for P and expressed C in milligrams per milliliter of urine.

Vorzimer, Cohen, and Joskow[1, 7] reported that they made the actual pigment reading on a Coleman Junior in terms of per cent transmission and then converted this to equivalent Klett reading by using the formula

$$\text{Klett} = 1739.05 - 867.75 \text{ (log Coleman)}.$$

Using P to represent this Klett reading, this simplifies to

$$P = 868 \text{ (2 - log \%T)} = 868 \text{ A}$$

where *%T* and *A* are, respectively, the per cent transmission and absorbance readings obtained in the Coleman.

This factor of 868 can be incorporated into the formula for calculating B.M.R. from P/C; thus,

$$0.25 \text{ P/C} = 0.25 \times 868 \text{ A/C} = 217 \text{ A/C}.$$

The estimation of basal metabolic rate from the P/C ratio, while not as reliable as one carefully made from oxygen consumption, may be of value when it is impossible to obtain an accurate measurement of oxygen consumption in the basal state.

## SUMMARY

A coefficient of correlation of only 0.36 was obtained in a series of forty-six simultaneous determinations of urinary pigment : creatinine ratio and of basal metabolic rate by oxygen consumption. The B.M.R. calculated from the P/C ratio agreed qualitatively with that calculated from oxygen consumption

in 61 per cent of the observations. The P/C B.M.R. corresponded with the clinical status in 62 per cent of all our observations and in 71 per cent of those on euthyroid and hyperthyroid patients. The $O_2$ B.M.R. corresponded with the clinical status in 81 per cent of our observations, on either basis. The P/C B.M.R. corresponded with the iodine uptake in eight of fifteen patients (53 per cent).

We are indebted to Mr. James R. Daniel who made the oxygen B.M.R. determinations and collected the urine specimens.

REFERENCES

1. Cohen, I. B.: Personal communication, 1951.
2. Drabkin, D. L.: The Normal Pigment of Urine. II. The Relationship of the Basal Metabolism to the Output of the Normal Urinary Pigment, J. Biol. Chem. **75**: 481-487, 1927.
3. Friedman, J., Marcus, H. R., and Vorzimer, J. J.: Prolonged Clinic Study of the Treatment of Hyperthyroidism, New York J. Med. **53**: 2807-2810, 1953.
4. Ostow, M., and Philo, S.: The Chief Urinary Pigment. The Relationship Between the Rate of Excretion of the Yellow Urinary Pigment and the Metabolic Rate, Am. J. M. Sc. **207**: 507-512, 1944.
5. Vorzimer, J. J., and Cohen, I. B.: Further Observations on the Use of the Urinary Pigment-Creatinine Ratio for the Measurement of Basal Metabolic Rate, J. LAB. & CLIN. MED. **34**: 1512-1515, 1949.
6. Vorzimer, J. J., Cohen, I. B., and Joskow, J.: The Use of Urinary Pigment Excretion for the Measurement of Basal Metabolic Rate, J. LAB. & CLIN. MED. **34**: 482-486, 1949.
7. Vorzimer, J. J., Cohen, I. B., and Joskow, J.: Further Observations on the Use of the Urinary Pigment-Creatinine Ratio for the Measurement of Basal Metabolic Rate, J. LAB. & CLIN. MED. **41**: 220, 1953.

# GLYCINE AND SYNTHESIS OF PARA-AMINOHIPPURIC ACID IN "FREE ANXIETY" AND HYPERTHYROIDISM

MARC J. MUSSER, M.D., WILLIAM P. DEISS, JR., M.D., AND THOMAS H. LORENZ, M.D., MADISON, WIS.

WITH THE TECHNICAL ASSISTANCE OF MARIA M. MOORE, B.S.

## INTRODUCTION

IT HAS been shown that patients having long-standing "free anxiety" have an increased excretion of hippuric acid following the intravenous administration of sodium benzoate.[1] Similar alterations in hippuric acid excretion were not observed in normal subjects under acute emotional stress, in patients in whom chronic anxiety was "bound" by conversion reactions or psychosomatic disorders, or in those with certain psychoses.[2] Comparable studies in patients suffering from hyperthyroidism, the clinical symptomatology of which sometimes resembles that of severe "free anxiety," have shown an abnormally low excretion of hippuric acid.[3]

Since the synthesis of hippuric acid is dependent not only upon the conjugating function of the liver, but also upon the availability of glycine, it is possible that some alteration in glycine metabolism could account for the observed alterations in hippuric acid excretion. Observations by Alexander[4] showing an increased hippuric acid synthesis after glycine administration lend support to this possibility.

The mechanism of para-aminohippuric acid (PAH) synthesis appears to be similar to that of hippuric acid, and it is influenced in the same manner by glycine.[5] An increase in PAH synthesis in two patients with severe, chronic anxiety reactions has been observed previously.[5] The initial phase of the present investigation was to confirm this by study of a larger group of similar patients. There is little direct evidence to support the view that the rather complex conjugating processes involved in this synthesis are altered in chronic "free anxiety" or in hyperthyroidism uncomplicated by liver disease. Thus, the present investigation was primarily designed to determine whether an alteration in glycine availability might account for the observed abnormalities in PAH synthesis in these two disorders. Serum glycine levels, both fasting and after a test dose of sodium para-aminobenzoate (sodium PAB), were measured, and the effect of glycine administration upon PAH synthesis was studied in an effort to appraise this possibility.

## MATERIALS AND METHODS

Three groups of subjects were studied. In none of these individuals was there clinical or laboratory evidence of hepatic or renal disease.

*Group I:* Seventeen healthy medical students and hospital staff members.

From the Department of Medicine, University Hospitals and the University of Wisconsin Medical School, Madison, Wis.

This investigation was supported in part by the Bess G. Heath Medical Research Fund.
Received for publication Sept. 20, 1954.

*Group II:* Twenty-two patients in whom a diagnosis of hyperthyroidism was established by clinical, laboratory, and radioactive iodine tracer studies.

*Group III:* Twenty-three patients with emotional disorders in whom chronic, severe "free anxiety" predominated, and who showed an abnormally increased PAH synthesis. "Free anxiety" has been described by Persky and associates[6] as a "condition of exaggerated foreboding or apprehension subjectively experienced by the patient and objectively observable by the presence of reactions to stress in the form of dilated pupils, warm perspiring skin, tachycardia, tremor, hyperpnoea, in attacks of exaggerated anxiety or panic, insomnia, restlessness, etc. Sufferers from "free anxiety" seem unable to protect themselves sufficiently from its horrible sensations by the well-known psychological defenses such as compulsions, phobias, conversions, displacements, acting out of flight or attack, etc."

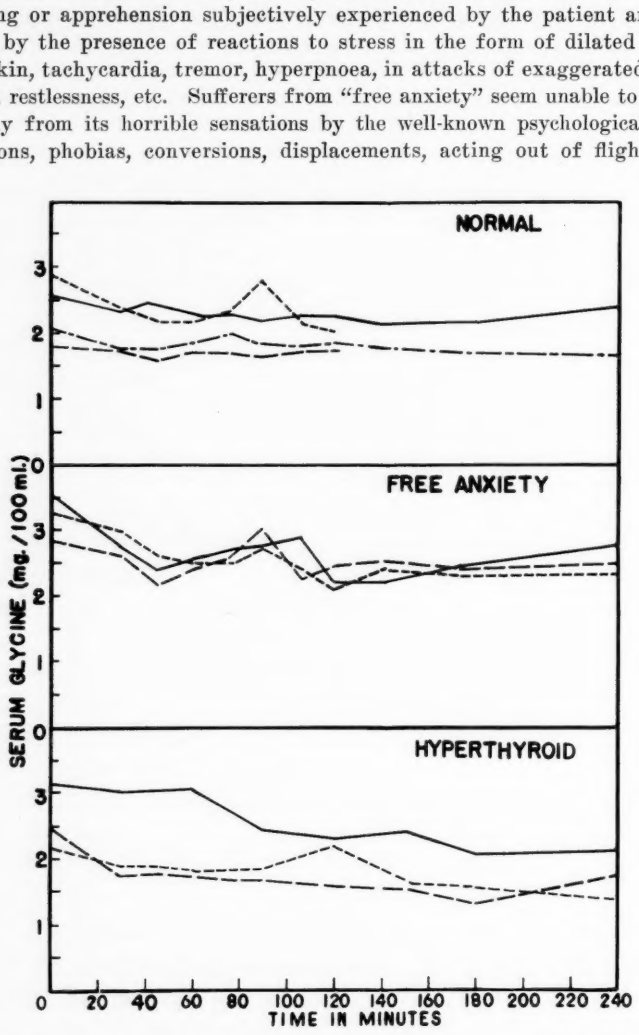



Fig. 1.—Serum glycine levels at timed intervals after an oral dose of sodium PAB given at "0" time.

In representative members of each group, serum glycine levels were determined colorimetrically by the method of Alexander[7] on samples taken after twelve hours of fasting and at timed intervals up to four hours following the ingestion of 6 Gm. of sodium PAB.

The effect of glycine administration upon PAH synthesis was studied in the following manner: one hour after an oral dose of 3 Gm. of sodium PAB, serum samples were obtained from each subject and analyzed colorimetrically for PAH according to the method of Deiss and Cohen.[5] Twenty-four hours later, PAH determinations were again made one hour after the ingestion of 3 Gm. of sodium PAB and 5 Gm. of glycine, U. S. P. All PAH values were expressed as the percentage of normal mean PAH synthesis.

## RESULTS

*PAH Synthesis.*—In the three groups of subjects studied, PAH synthesis was found to parallel the previously reported alterations in hippuric acid excretion.[1] Compared to the mean of the seventeen normal subjects, taken as 100 per cent, the average synthesis of PAH was 151 per cent in the twenty-three selected "free anxiety" subjects and 71 per cent in the twenty-two patients with hyperthyroidism (Fig. 1).

*Serum Glycine Levels.*—In Table I are tabulated the fasting levels of serum glycine in representative subjects of each of the three groups. In several instances, determinations were made on consecutive days. In the limited number of specimens studied the mean serum glycine level in the "free anxiety" subjects was slightly higher, and in the hyperthyroid patients, slightly lower, than in the normal. The small differences in the means, as well as the intragroup variability of these determinations, do not permit a positive conclusion. Serum glycine levels during a four-hour period following the ingestion of 6 Gm. of sodium PAB in four normal subjects, three patients with "free anxiety," and three hyperthyroid patients are illustrated in Fig. 2. No striking fluctuations in the serum glycine levels were noted.

TABLE I. FASTING SERUM GLYCINE LEVELS

| SUBJECTS | SERUM GLYCINE IN MG./100 ML. | MEAN |
|---|---|---|
| *Normal* | | 2.65 |
| D. W. | 2.35 | |
| | 2.05 | |
| L. T. | 2.85 | |
| | 2.93 | |
| M. J. | 2.55 | |
| | 2.96 | |
| M. M. | 2.70 | |
| | 2.83 | |
| *Hyperthyroid* | | 2.21 |
| W. W. | 2.13 | |
| | 2.43 | |
| W. S. | 2.23 | |
| A. S. | 1.25 | |
| T. S. | 3.03 | |
| *Free Anxiety* | | 3.07 |
| A. R. | 2.87 | |
| S. D. | 3.50 | |
| T. N. | 2.97 | |
| R. C. | 3.20 | |
| | 2.82 | |

*Effect of Glycine Administration on PAH Synthesis.*—Following the ingestion of glycine there was an increase in PAH synthesis in the normal group to a level of 162 per cent of the original mean. This increase in synthesis after glycine in the normal group is compared in Fig. 3 with the increase in the other two groups under similar conditions of glycine administration. There was an increase in synthesis from 71 per cent to 158 per cent of the original normal mean in the hyperthyroid group, and an increase from 154 per cent to 194 per cent of the original normal mean in the "free anxiety" subjects. It

will be noted that the PAH synthesis following glycine administration in the hyperthyroid patients was increased 120 per cent, so that quantitatively it became comparable to that in the normal subjects fed glycine. On the other hand, the increase of PAH synthesis in the "free anxiety" group was only 26 per cent.

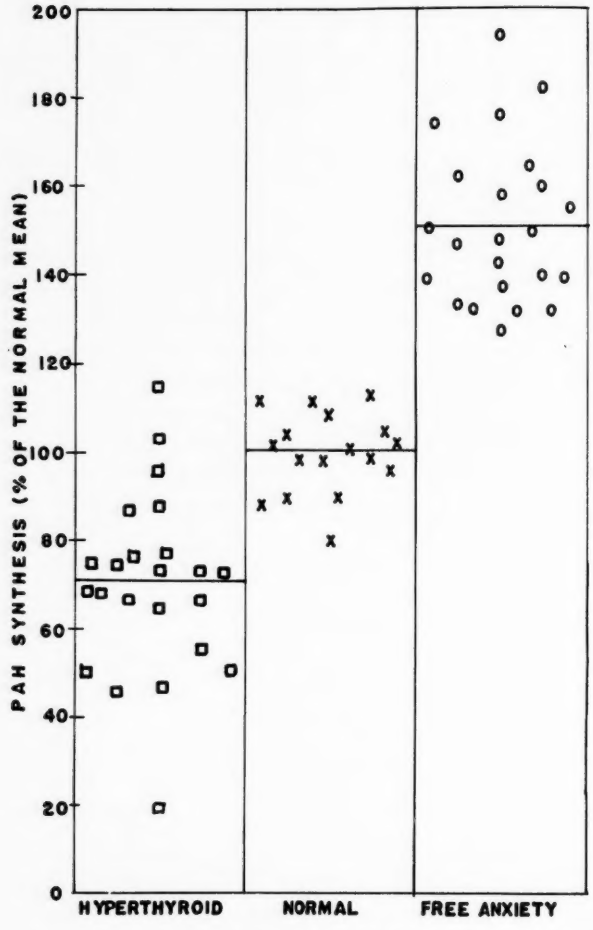



Fig. 2.—Comparison of PAH synthesis in the three groups. The horizontal lines represent the mean of each group.

## DISCUSSION

It is clear both from these data and previous observations that there is a definite and striking increase in hippuric acid and PAH synthesis in certain patients suffering from chronic "free anxiety." The reason for this abnormality is not clear. Any one of a sequence of biochemical processes in the synthesis of hippuric acid or PAH could be responsible. One possibility is that in the presence of chronic "free anxiety" an increased availability and/or mobilization of glycine occurs. The slight increase over the normal in fasting serum glycine levels which was noted is compatible with such an hypoth-

esis. Furthermore, the relatively small increase in PAH synthesis in these patients (26 per cent) after the administration of glycine might be interpreted as indicating that an excess of endogenous glycine was already available.

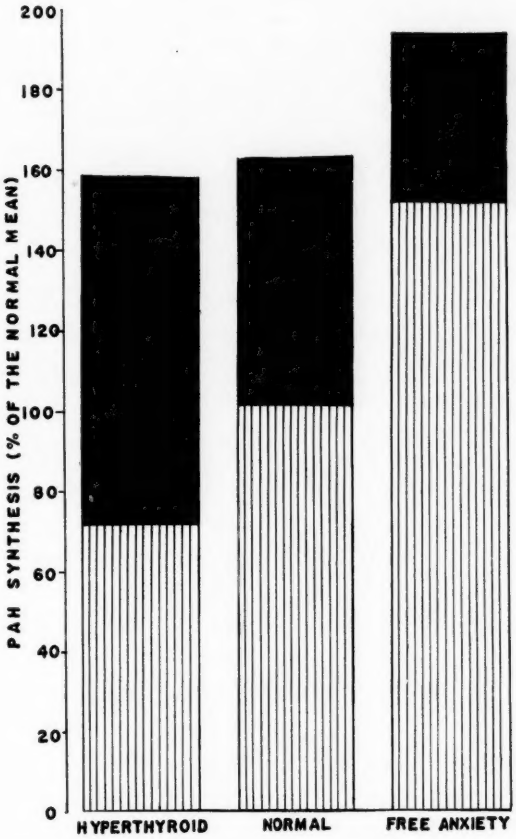



Fig. 3.—Bar graph showing the increase in PAH synthesis following glycine administration in the three groups. The black segments of the bars represent the mean increase in synthesis over the original means, indicated by the lined segments.

The significance of glycine availability becomes even more apparent in the results of the studies of hyperthyroid patients. It has been suggested that subnormal hippuric acid excretion in hyperthyroidism is a reflection of liver damage.[3] The restoration of PAH synthesis to normal by the administration of glycine, as seen in Fig. 3, suggests that the defect is not in the conjugating mechanism as is characteristic in most instances of actual liver disease,[5] but is rather a reflection of a depletion of available glycine. That glycine stores should become depleted in hyperthyroidism is entirely reasonable in the face of the tremendous metabolic demands which occur in this disease. Again, the subnormal fasting serum glycine levels in the few hyperthyroid subjects studied are consistent with such a phenomenon.

SUMMARY

The synthesis of PAH in twenty-three subjects with chronic "free anxiety" was distinctly increased (151 per cent of the normal mean) and in twenty-two patients with hyperthyroidism was decreased (71 per cent of the normal mean). Following the administration of glycine, the increase in PAH synthesis was much more pronounced in the hyperthyroid group than in the normal or chronic "free anxiety" groups. The reduced level of PAH synthesis and the marked increase in synthesis following glycine ingestion in the hyperthyroid subjects are believed to be reflections of a decreased availability of endogenous glycine in these patients. Conversely, the increased PAH synthesis and the relatively small increase in synthesis after glycine ingestion are believed to be reflections of increased availability and/or mobilization of endogenous glycine in certain patients with chronic "free anxiety."

REFERENCES

1. Persky, H., Grinker, R. R., and Mirsky, I. A.: The Excretion of Hippuric Acid in Subjects With Free Anxiety, J. Clin. Invest. **29**: 110-114, 1950.
2. Persky, H., Gamm, S. R., and Grinker, R. R.: Correlation Between Fluctuation of Free Anxiety and Quantity of Hippuric Acid Excretion, Psychosom. Med. **14**: 34-40, 1952.
3. Bartels, E. C., and Perkins, H. J.: Liver Function in Hyperthyroidism as Determined by the Hippuric Acid Test, New England J. Med. **216**: 1051-1060, 1937.
4. de Vries, A., and Alexander, B.: Studies on Amino Acid Metabolism. III. Plasma Glycine Concentration and Hippuric Acid Formation Following the Ingestion of Benzoate, J. Clin. Invest. **27**: 665-668, 1948.
5. Deiss, W. P., and Cohen, P. P.: Studies in Para-Aminohippuric Acid Synthesis in the Human: Its Application as a Liver Function Test, J. Clin. Invest. **29**: 1014-1020, 1950.
6. Persky, H., Grinker, R. R., Mirsky, I. R., and Gamm, S. R.: Life Situations, Emotions, and the Excretion of Hippuric Acid in Anxiety States, Proc. A. Res. Nerv. & Ment. Dis. **29**: 297-306, 1950.
7. Alexander, B., Landwehr, G., and Seligman, A. M.: A Specific Micromethod for the Colorimetric Determination of Glycine in the Blood and Urine, J. Biol. Chem. **160**: 51-59, 1945.

# SERUM AND LIVER LIPIDS IN PATIENTS WITH AND WITHOUT LIVER DISEASE

BARBARA H. BILLING, PH.D., RUTH M. HASLAM, M.D., DAVID E. HEIN, M.D., HAROLD J. CONLON, M.D., DAVID L. HAMILTON, M.D., GORDON M. MINDRUM, M.D., AND LEON SCHIFF, M.D., PH.D.
CINCINNATI, OHIO

WITH THE TECHNICAL ASSISTANCE OF LILA DOHM, B.S., AND CHARLENE STARR, B.S.

## INTRODUCTION

THIS study has been undertaken to determine the relationship of serum and liver lipids. Man and associates[1] studied the serum lipid fractions in seventy cases of liver disease. In nine of their cases a determination of the liver lipids in autopsy material also was made. The authors did not discuss their findings, but it does not appear that there was any association. Steuck and co-workers[2] studied the liver and serum lipids in twenty-one subjects (nineteen cirrhotics and two noncirrhotics) and concluded that there is no absolute parallelism between them. The liver lipids were determined post mortem and the serum lipids in samples obtained from one to as many as 254 days before death. Thannhauser and Reinstein[3] reported serum lipid values and post-mortem liver and spleen lipid values in a large variety of diseases. No time relationship of the serum values to autopsy was given. They stated that hyperlipemia may occur with normal liver lipids. Beveridge and Johnson,[4] in a study on the effect of alloxan diabetes and diet in rats, found that for both fatty acids and total cholesterol a high level in the plasma was accompanied by a high level in the liver.

In the present study chemical analyses were carried out on eighty-five specimens of liver obtained by needle biopsy for diagnostic purposes. It has previously been shown by Billing and colleagues[5] that, within certain limits, a single biopsy sample is representative of the liver as a whole in respect to its lipid content. Determinations of serum lipids were made at the same time and their relation to liver lipids investigated.

## MATERIALS AND METHODS

*Cases.*—A total of 82 cases were investigated and 85 observations were made. The cases were classified on the basis of the histologic diagnosis made on the biopsy specimen. Of these cases 20 showed no liver abnormality. One of this group (E. H.) was a case of congenital hyperlipemia with xanthomatosis; because of the very abnormal serum lipid values the results were not included for statistical analysis.

From the Department of Internal Medicine, College of Medicine, University of Cincinnati, and the Gastric Laboratory, Cincinnati General Hospital.

This work was aided by grants from the Robert Gould Research Foundation, the Milton Brown Memorial Fund, the John Eisman Memorial Fund, and Fred Weiland.

Received for publication Oct. 4, 1954.

Twenty-three cases of cirrhosis were studied; of these, 10 (11 observations) were classed as being of the nutritional type,[6] 7 as portal cirrhosis of undetermined origin, 2 as postnecrotic, and 1 as biliary cirrhosis. Three cases showed portal area fibrosis and are included.

There were 12 cases of viral hepatitis, in 10 of which determinations were made between 1 and 3 weeks after the onset of symptoms, while in the 2 remaining cases with chronic active disease, the studies were made at 3 and 10 months, respectively.

TABLE I. SERUM LIPID VALUES IN HEALTHY SUBJECTS

| LIPID FRACTION | RANGE | MEAN | S.D. | NO. OF OBSERVATIONS |
|---|---|---|---|---|
| *Male subjects* | | | | |
| Total lipid | 481-980 | 704 | 113 | 22 |
| Total fatty acid | 349-615 | 438 | 76 | 22 |
| Phospholipid | 186-306 | 247 | 33 | 39 |
| Total cholesterol | 127-296 | 193 | 39 | 39 |
| Cholesterol ester | 95-211 | 138 | 26 | 39 |
| Neutral fat | 104-321 | 183 | 62 | 22 |
| *Female subjects* | | | | |
| Total lipid | 387-885 | 598 | 102 | 23 |
| Total fatty acid | 208-516 | 331 | 64 | 23 |
| Phospholipid | 188-322 | 249 | 30 | 51 |
| Total cholesterol | 119-272 | 186 | 37 | 51 |
| Cholesterol ester | 91-193 | 136 | 26 | 51 |
| Neutral fat | 15-182 | 60 | 44 | 23 |

TABLE II. SERUM AND LIVER LIPIDS IN CASES WITH HISTOLOGICALLY NORMAL LIVER BIOPSY SPECIMEN

| SEX | CASE | CLINICAL DIAGNOSIS | SERUM LIPIDS (MG./100 ML.) | | | | | | LIVER LIPID (GM./100 GM. WET LIVER) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TOTAL LIPID | TOTAL FATTY ACID | NEUTRAL FAT | PHOSPHOLIPID | CHOLESTEROL TOTAL | CHOLESTEROL ESTER | TOTAL LIPID | TOTAL FATTY ACID | PHOSPHOLIPID |
| M | R. A. | Pneumonia | 672 | 407 | 164 | 223 | 190 | 132 | 5.5 | 3.3 | 3.6 |
| F | S. R. | Carcinoma of gall bladder | 665* | – | – | 199 | 141 | 97 | 4.6 | – | – |
| M | C. D. | Alcoholism | 673 | 394 | 122 | 250 | 198 | 146 | 6.2 | – | 4.0 |
| F | P. T. | Pulmonary tuberculosis | 669 | 399 | 171 | 205 | 198 | 131 | 3.9 | – | – |
| F | D. R. | Multiple myeloma | 703 | 474 | 262 | 207 | 155 | 110 | 4.9 | 4.5 | 2.8 |
| F | C. R. | Recent infectious hepatitis | 846 | 485 | 148 | 320 | 257 | 160 | 4.8 | 3.1 | 2.4 |
| M | J. I. | Alcoholism | 874 | 502 | 201 | 224 | 306 | 213 | 4.8 | 3.6 | 3.5 |
| M | J. H. | Alcoholism | 773 | 496 | 249 | 231 | 196 | 134 | 6.3 | – | – |
| F | V. W. | Postmeasles pneumonia | 629 | 419 | 222 | 196 | 140 | 98 | 5.5 | – | – |
| M | J. W. | Congestive failure | 826 | 547 | 183 | 272 | 182 | 123 | 6.0 | 3.6 | 3.3 |
| F | G. B. | Psychoneurosis | 1,078 | 648 | 253 | 370 | 305 | 209 | 6.5 | 3.7 | 3.2 |
| M | P. P. | Hematemesis—cause unknown | 917 | 572 | 276 | 260 | 254 | 176 | 6.1 | 3.9 | 3.2 |
| F | B. D. | Pancreatic cyst | 640 | 376 | 88 | 295 | 171 | 120 | 4.9 | 3.6 | 3.6 |
| M | G. R. | Tuberculous lymphadenitis | 783 | 395 | 30 | 330 | 283 | 194 | 3.1 | 2.0 | 2.8 |
| M | R. McC. | Alcoholism | 1,218 | 717 | 222 | 504 | 347 | 248 | 2.6 | – | – |
| F | E. B. | Kimmelstiel-Wilson disease | 961 | 672 | 395 | 242 | 212 | 156 | 5.7 | 2.8 | 2.6 |
| F | E. K. | Hepatomegaly—cause unknown | 1,112 | 775 | 477 | 305 | 224 | 148 | 5.6 | 4.2 | 2.2 |
| M | L. W. | Secondary syphilis | 588 | 368 | 139 | 228 | 143 | 108 | 4.9 | – | – |
| M | C. E. | Secondary syphilis | 565* | – | – | 265 | 222 | 165 | 3.2 | – | – |
| F | E. H. | Xanthomatosis | 1,764 | 871 | 305 | 560 | 607 | 406 | 8.3 | 5.5 | 2.6 |

*Turbidimetric method.

TABLE III. SERUM AND LIVER LIPIDS IN HEPATIC CIRRHOSIS

| SEX | CASE | TYPE OF CIRRHOSIS | SERUM LIPIDS (MG./100 ML.) TOTAL LIPID | TOTAL FATTY ACID | NEUTRAL FAT | PHOSPHOLIPID | CHOLESTEROL TOTAL | CHOLESTEROL ESTER | LIVER LIPID (GM./100 GM. WET LIVER) TOTAL LIPID | TOTAL FATTY ACID | PHOSPHOLIPID |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M | W. W. | Nutritional | 271 | 177 | 84 | 120 | 54 | 18 | 3.0* | - | - |
| M | A. R. | Nutritional | 712 | 446 | 169 | 275 | 185 | 130 | 18.0 | 12.7 | 2.5 |
| F | E. M. | Nutritional | 414 | 263 | 113 | 159 | 97 | 62 | 1.5 | - | - |
| F | L. H. | Nutritional | 749 | 467 | 199 | 260 | 195 | 133 | 9.6 | - | - |
| F | J. M. | Nutritional | 1,058 | 617 | 224 | 362 | 316 | 218 | 20.9 | 19.1 | 1.9 |
| F | R. D.† | Nutritional | 924 | 576 | 246 | 405 | 213 | 83 | 27.0 | 24.6 | 3.5 |
| F | E. S. | Nutritional | 876 | 527 | 205 | 260 | 259 | 172 | 2.7 | - | - |
| M | L. F. | Nutritional | 628 | 355 | 80 | 313 | 172 | 87 | 19.5 | 18.5 | 3.1 |
| M | A. B. | Nutritional | 833 | 526 | 242 | 258 | 218 | 160 | 10.2 | 7.8 | 2.6 |
| F | B. A. | Nutritional | 708 | 440 | 195 | 262 | 180 | 100 | 11.2 | - | 3.8 |
| F | B. A. | Nutritional | 783 | 513 | 273 | 277 | 223 | 140 | 4.4 | - | - |
| M | J. B. | Portal | 745‡ | - | - | 257 | 178 | 122 | 5.1 | - | - |
| M | N. B. | Portal | 283 | 184 | 56 | 154 | 49 | 32 | 2.1 | - | - |
| M | H. G. | Portal | 665 | 434 | 215 | 227 | 142 | 99 | 11.8 | - | 2.8 |
| F | D. K. | Portal | 646 | 462 | 293 | 178 | 117 | 81 | 5.3 | 3.5 | 3.3 |
| F | M. P. | Portal | 665 | 430 | 241 | 183 | 169 | 101 | 6.5 | 4.1 | 3.0 |
| F | M. K. | Biliary | 874 | 543 | 234 | 285 | 234 | 169 | 2.9 | - | - |
| M | C. F. | Portal fibrosis | 659 | 403 | 162 | 223 | 181 | 129 | 7.5 | 6.7 | 3.8 |
| M | W. M. | Portal fibrosis | 743 | 431 | 133 | 280 | 220 | 152 | 11.1 | - | - |
| M | C. D. | Portal fibrosis | 548 | 328 | 123 | 193 | 155 | 107 | 5.6 | 3.7 | 3.6 |
| F | M. S. | Postnecrotic | 616 | 399 | 194 | 198 | 147 | 104 | 4.5 | 1.4 | 2.0 |
| M | B. L. | Postnecrotic | 567 | 371 | 175 | 204 | 125 | 84 | 4.0 | 1.9 | 1.8 |
| F | E. R. | Biliary | 1,376 | 775 | 264 | 522 | 430 | 246 | 4.8 | 3.2 | 3.8 |

*Post-mortem biopsy.
†Previous result on this patient included in fatty vacuolization group.
‡Turbidimetric method.

TABLE IV. SERUM AND LIVER LIPIDS IN HEPATITIS

| SEX | CASE | TYPE OF HEPATITIS | SERUM LIPIDS (MG./100 ML.) TOTAL LIPID | TOTAL FATTY ACID | NEUTRAL FAT | PHOSPHOLIPID | CHOLESTEROL TOTAL | CHOLESTEROL ESTER | LIVER LIPID (GM./100 GM. WET LIVER) TOTAL LIPID | TOTAL FATTY ACID | PHOSPHOLIPID |
|---|---|---|---|---|---|---|---|---|---|---|---|
| F | A. W. | Viral | 1,160 | 732 | 329 | 538 | 250 | 62 | 4.4 | 2.5 | 2.6 |
| F | D. W. | Viral | 837 | 565 | 324 | 280 | 173 | 84 | 4.4 | - | 3.0 |
| F | O. S. | Viral | 384 | 264 | 147 | 163 | 62 | 15 | 3.6 | 1.9 | 2.40 |
| M | J. M. | Viral | 922 | 577 | 253 | 368 | 230 | 98 | 7.2 | 3.9 | 3.3 |
| M | H. K. | Viral | 956 | 574 | 232 | 418 | 243 | 88 | 4.7 | - | - |
| M | J. H. | Viral | 855 | 539 | 247 | 319 | 218 | 113 | 3.3 | - | - |
| M | V. E. | Viral | 542 | 308 | 75 | 204 | 168 | 132 | 2.8 | - | - |
| F | E. D. | Viral | 727 | 497 | 286 | 256 | 141 | 63 | 3.7 | - | - |
| M | C. B. | Viral | 732 | 496 | 261 | 242 | 151 | 100 | 4.1 | 2.6 | 2.8 |
| M | H. F. | Viral | 914 | 596 | 311 | 400 | 182 | 28 | 5.7 | - | - |
| M | R. B. | Viral | 674 | 444 | 227 | 216 | 154 | 108 | 4.2 | - | 2.4 |
| F | J. T. | Viral | 662 | 409 | 156 | 250 | 169 | 120 | 6.2 | 2.9 | 3.0 |
| M | E. G. | Focal acute nonspecific | 671 | 422 | 173 | 228 | 171 | 125 | 4.2 | 2.7 | 2.2 |
| F | D. C. | Focal acute nonspecific | 550 | 322 | 107 | 204 | 160 | 109 | 4.7 | 3.2 | 3.1 |
| F | A. B. | Focal acute nonspecific | 797 | 478 | 148 | 312 | 216 | 106 | 4.4 | 4.4 | 3.5 |
| F | J. M. | Active chronic viral | 819 | 493 | 168 | 310 | 224 | 162 | 4.4 | 3.2 | 3.2 |
| F | M. S. | Active chronic viral | 739 | 429 | 161 | 240 | 230 | 151 | 5.4 | 3.0 | 3.4 |
| F | M. B. | Toxic | 997 | 584 | 206 | 490 | 237 | 61 | 2.4 | 1.9 | 2.2 |
| M | J. C. | Mild* | 488 | 292 | 99 | 190 | 133 | 92 | 7.2 | - | 2.4 |

*Consistent with, but not diagnostic of, viral hepatitis.

TABLE V. SERUM AND LIVER LIPIDS IN MISCELLANEOUS HEPATIC DISORDERS

| SEX | CASE | HISTOLOGIC DIAGNOSIS | SERUM LIPIDS (MG./100 ML.) | | | | | | LIVER LIPID (GM./100 GM. WET LIVER) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TOTAL LIPID | TOTAL FATTY ACID | NEUTRAL FAT | PHOSPHOLIPID | CHOLESTEROL | | TOTAL LIPID | TOTAL FATTY ACID | PHOSPHOLIPID |
| | | | | | | | TOTAL | ESTER | | | |
| F | B. O. | Fatty vacuolization | 716 | 430 | 205 | 260 | 197 | 149 | 6.2 | 4.2 | 2.9 |
| M | J. Z. | Fatty vacuolization | 966 | 295 | 241 | 144 | 286 | 200 | 9.1 | 6.4 | 2.1 |
| M | A. P. | Fatty vacuolization | 549 | 362 | 175 | 196 | 124 | 82 | 6.4 | - | 2.8 |
| M | H. B. | Fatty vacuolization | 947 | 597 | 301 | 258 | 258 | 181 | 10.6 | - | 3.7 |
| F | R. D. | Fatty vacuolization | 784 | 492 | 226 | 255 | 216 | 138 | 33.5 | - | 3.1 |
| M | R. C. | Fatty vacuolization | 561 | 335 | 102 | 219 | 153 | 120 | 13.2 | - | 3.4 |
| M | W. J. | Fatty vacuolization | 685 | 426 | 181 | 235 | 179 | 125 | 9.4 | 8.3 | 2.6 |
| M | C. R. | Focal necrosis nonspecific | 869 | 539 | 223 | 330 | 218 | 86 | 5.5 | 3.6 | 2.8 |
| M | J. G. | Focal necrosis nonspecific | 814 | 532 | 316 | 228 | 180 | 127 | 6.3 | 5.6 | 4.0 |
| F | C. G. | Focal necrosis nonspecific | 501 | 334 | 149 | 213 | 95 | 62 | 10.9 | 8.4 | 2.2 |
| M | F. S. | Focal necrosis nonspecific | 618 | 366 | 115 | 250 | 171 | 115 | 3.8 | 2.9 | 2.5 |
| M | O. C. | Focal necrosis nonspecific | 486 | 272 | 51 | 210 | 147 | 109 | 2.6 | 2.4 | 2.9 |
| M | R. W. | Focal necrosis nonspecific | 1,116 | 759 | 472 | 402 | 216 | 38 | 7.4 | - | - |
| F | K. B. | Granuloma | 681 | 386 | 117 | 250 | 222 | 163 | 12.0 | - | - |
| M | E. B. | Granuloma | 473 | 308 | 105 | 198 | 140 | 98 | 3.6 | - | - |
| F | T. W. | Focal necrosis nonspecific | 762 | 468 | 188 | 250 | 230 | 159 | 3.2 | - | - |
| M | L. M. | Granuloma | 617 | 411 | 211 | 212 | 133 | 85 | 6.6 | 3.6 | 3.6 |
| M | J. B. | Focal necrosis nonspecific | 973 | 677 | 382 | 355 | 237 | 81 | 9.5 | 8.0 | 3.0 |
| M | J. B. | Granuloma | 563 | 356 | 145 | 218 | 134 | 93 | 4.0 | 2.9 | - |
| F | N. S. | Metastatic carcinoma | 632 | 417 | 206 | 220 | 139 | 93 | 3.1 | 1.4 | 1.6 |
| M | R. C. | Metastatic carcinoma | 542 | 345 | 234 | 228 | 121 | 79 | 4.4 | 2.8 | - |
| M | J. K. | Metastatic carcinoma | 660 | 404 | 166 | 235 | 176 | 114 | 2.7 | - | - |
| M | O. W. | Lymphoma | 837 | 482 | 170 | 270 | 265 | 184 | 4.8 | - | - |

Seven cases of nonspecific or toxic hepatitis, 5 cases of nonspecific focal necrosis, 8 cases of fatty vacuolization without cirrhosis, 5 cases (6 observations) of granuloma, 3 cases of metastatic carcinoma, and 1 case of lymphoma were also studied.

In Cases B. A., nutritional cirrhosis, and J. B., granuloma, 2 observations were made, 2 weeks and 2 months apart, respectively. Both sets of results have been included in the statistical analysis. In Case R. D. 2 observations were made 6 months apart; the first biopsy showed fatty vacuolization, the second nutritional cirrhosis. The results have been included in the respective groups as separate cases.

To obtain a series of normal values for serum lipid fractions by the methods used in this study, determinations were made on serum from a number of healthy young adults (medical students, nurses, and laboratory staff members). The results are recorded in Table I.

*Methods.*—The serum for analysis was obtained in nearly all instances within two days of the liver biopsy. Serum was drawn with the patient in the fasting state.

The following determinations on serum were made: total fatty acids by a modification of the method of Stoddart and Drury;[7] total, free, and ester cholesterol by the method of Sperry and Webb.[8] Phospholipids were determined by measurements of the phosphate after extraction with Bloors' reagent and digestion with 5 N $H_2SO_4$, using molybdate reduction reaction with 0.5 per cent methyl aminophenyl sulfate in 3 per cent sodium bisulfite; the lipid phosphorus content was multiplied by 25 to give the value for phospholipids. Neutral fat and total lipids were determined by calculation.[3] In 3 cases where the estimation of total fatty acids was omitted the total serum lipid was determined by the turbidimetric method of Kunkel and associates.[9] All results are expressed as milligrams per 100 ml. of serum.

A total lipid determination was made in all the liver specimens; in a considerable number of cases total fatty acids and phospholipids were also determined. The weighing of the biopsy sample, its subsequent treatment, and the chemical methods employed were those described by Billing and collaborators.[5] All the results are expressed as gram lipid per 100 Gm. wet liver tissue.

## RESULTS

It is not the purpose of this paper to discuss the individual findings or the abnormalities in any of the serum or liver lipid components in relation to the liver pathology. The results are fully tabulated (Tables II to V) and only brief and general comments will be made in so far as they relate to the aspect of the study under consideration; namely, the relationship between serum and liver lipids whether or not the values fall within the normal range.

In each group of cases the dependence of the liver lipid upon the serum lipid level was determined. Because of the nature of the distributions, logarithmic values for the liver lipid were used in calculating the regressions. In each group an analysis was made with respect to the total lipids of serum and liver. The relation between the different serum lipid fractions and the total liver lipid was studied in the larger groups. The results of the analyses are recorded in Table VI. Unless otherwise stated, the total liver lipid calculated on a wet weight basis was used. The sign of the figure "b" (regression coefficient) indicates the trend toward positive (direct) or negative (inverse) association. The per cent of variance accounted for by the regression indicates the strength of the association. A value of 0.05 for P is taken as the accepted limit of significance.

TABLE VI. ANALYSIS OF RESULTS, DEPENDENCE OF TOTAL LIVER LIPID ON SERUM LIPIDS

| GROUP | SERUM COMPONENT | b (REGRESSION COEFFICIENT) | PER CENT OF VARIANCE ACCOUNTED FOR BY REGRESSION | NO. OF OBSERVATION | P |
|---|---|---|---|---|---|
| Portal cirrhosis | Total lipid | +0.010 | 31 | 22 | 0.01-0.001 |
| | | (+0.0069)* | (22) | (22) | (0.05-0.02) |
| | | (+0.0093)† | (23) | (22) | (0.05-0.02) |
| | Total fatty acid | +0.016 | 29 | 21 | 0.02-0.01 |
| | Neutral fat | +0.014 | 6 | 21 | 0.3-0.2 |
| | Phospholipid | +0.037 | 54 | 22 | <0.001 |
| | Cholesterol total | +0.027 | 24 | 22 | 0.02 |
| | free | +0.080 | 35 | 22 | 0.01-0.001 |
| | ester | +0.025 | 12 | 22 | 0.2-0.1 |
| Normal liver | Total lipid | −0.000066 | <0.1 | 19 | >0.9 |
| | | (−0.00045)* | (<1) | (19) | (0.8-0.7) |
| | | (−0.00049)† | (<1) | (19) | (0.8-0.7) |
| | Total fatty acid | −0.00031 | 1 | 17 | 0.9 |
| | Neutral fat | +0.0037 | 14 | 17 | 0.2-0.1 |
| | Phospholipid | −0.0066 | 19 | 19 | 0.1-0.05 |
| | Cholesterol total | −0.0069 | 14 | 19 | 0.2-0.1 |
| Hepatitis, viral | Total lipid | +0.0023 | 17 | 12 | 0.2 |
| | | (+0.0026)* | (24) | (12) | (0.1) |
| | | (+0.0032)† | (24) | (12) | (0.1) |
| | Total fatty acid | +0.0038 | 18 | 12 | 0.2-0.1 |
| | Neutral fat | +0.0049 | 10 | 12 | 0.3 |
| | Phospholipid | +0.0044 | 17 | 12 | 0.2 |
| | Cholesterol total | +0.0073 | 11 | 12 | 0.3 |
| Fatty vacuolization | Total lipid | +0.000076 | 0 | 8 | >0.9 |
| | | (+0.0014)† | (<1) | (8) | (0.9-0.8) |
| Granuloma | Total lipid | +0.0067 | 24 | 6 | 0.4-0.3 |
| | | (+0.0084)† | (35) | (6) | (0.2) |
| Nonspecific focal necrosis | Total lipid | +0.0029 | 10 | 5 | 0.6 |

*Calculated on values for dry liver.
†Calculated on values for dry fat-free liver.

*Normal Liver.*—The total liver lipid values fell within a range of 2.6 to 8.3 Gm. per 100 Gm. wet liver with a mean of 5.2 Gm. per cent. These findings correspond closely with those of Ralli and associates,[10] who from a study of twenty-five normal livers give as a normal range for total liver fat 2.4 to 8.4 Gm. per 100 Gm. wet liver, with a mean value of 5.0 Gm. per cent.

Five of the twenty cases had abnormally high serum lipids and in four of these five the total liver lipid was above the mean value for the group. Statistical analysis (for nineteen cases, E. H. excluded) showed no significant association between the total liver lipid and the total serum lipid or any of the serum lipid fractions.

*Cirrhosis.*—The cases of portal cirrhosis were considered as one group. The highest liver lipid levels were found in the cases described as nutritional cirrhosis. Seven of these eleven observations showed abnormally high values ranging from 9.6 to 27.0 Gm. per cent. In the remaining cases of cirrhosis, only two of twelve cases had a high liver lipid. There were two cases in the group

with abnormally low serum lipids; these also had very low liver lipids. The one case with a high serum lipid also had a very high liver lipid.

There was a significant, but small, positive association between total liver lipids and total serum lipids, and also between total liver lipids and serum fatty acids, phospholipids, and total and free cholesterol. There was no significant association between total liver lipids and serum neutral fat and esterified cholesterol. On an average only 31 per cent of the variation in liver lipid can be accounted for by a variation in serum lipid. The liver lipid for a given serum lipid value in a case of cirrhosis cannot, therefore, be predicted from the calculated regression line.

*Viral Hepatitis.*—In all twelve cases of viral hepatitis the liver lipid was within the normal range; there was a tendency toward rather low values. The serum total lipid values tended to fall in the upper part of the normal range, but there was one low value and one abnormally high value. There was, however, no significant association between serum and liver lipids.

No analysis was made in the nonviral hepatitis cases since these formed a small and very mixed group.

*Other Groups.*—High liver lipid values were found in five of eight cases of fatty vacuolization (in the remaining three cases with a normal liver lipid, the fatty vacuolization was only slight). High liver lipid values were found also in one of five cases of nonspecific focal necrosis and in one of six observations on granuloma. No significant association was found between serum and liver total lipids in any of these groups.

It was thought that different and perhaps more significant results might be found if the analyses were applied using liver lipid values calculated on a dry weight or dry fat-free weight basis. In the groups other than cirrhosis, P values thus obtained were only slightly lower than the corresponding P values for the calculations on a liver wet-weight basis. In the cirrhosis group the P value was slightly higher in this analysis.

## SUMMARY AND CONCLUSIONS

Parallel determinations of serum and liver lipids have been made in a series of eighty-two patients with and without liver disease in whom a total of eighty-five observations were made. The liver samples were obtained by needle biopsy. In a group of nineteen cases with normal livers, statistical analysis showed no significant association between the total liver lipid and the serum total lipid or any of the serum lipid fractions. In a group of twenty-two cases of hepatic cirrhosis a positive association was found between the total liver lipid and serum total lipid, fatty acid, phospholipid, and total and free cholesterol. Although statistically significant this association is not a marked one and a measurement of the serum lipid in a case of cirrhosis could not be used to predict the liver lipid level. There was not a significant association between the total liver lipid and the serum lipids in a group of twelve cases of viral hepatitis, nor in the smaller groups of fatty vacuolization, focal necrosis, or granuloma.

The authors wish to acknowlege the valuable assistance of Dr. Henry Ryder in the statistical analysis of the data.

## REFERENCES

1. Man, E. B., Kartin, B. H., Durlacher, S. H., and Peters, J. P.: The Lipids of Serum and Liver in Patients With Hepatic Disease, J. Clin. Invest. **24**: 623-643, 1945.
2. Steuck, G. H., Jr., Rubin, S. H., Clarke, D. H., Graef, I., and Ralli, E. P.: Studies on Patients With Cirrhosis of the Liver: Plasma Liver Lipid Distribution and Its Relation to the Pathology of the Liver, Am. J. Med. **5**: 188-201, 1948.
3. Thannhauser, S. J., and Reinstein, H.: Fatty Changes in the Liver From Different Causes: Comparative Studies of the Lipid Partition, Arch. Path. **33**: 646-654, 1942.
4. Beveridge, J. M. R., and Johnson, S. E.: Studies on Diabetic Rats—The Effect of Diabetes and of Diet Upon the Plasma and Liver Lipids of Rats, Brit. J. Exper. Path. **31**: 294-305, 1950.
5. Billing, B. H., Conlon, H. J., Hein, D. E., and Schiff, L.: The Value of Needle Biopsy in the Chemical Estimation of Liver Lipids in Man, J. Clin. Invest. **32**: 214-225, 1953.
6. Schneider, E. M., Berman, J. R., Gall, E. A., and Schiff, L.: Needle Biopsy of the Liver. IV. Relationship of Clinical and Laboratory Findings to Histological Structure in 100 Cases of Portal Cirrhosis, Am. J. Med. **15**: 207-215, 1953.
7. Stoddart, J. L., and Drury, P. E.: A Titration Method for Blood Fat, J. Biol. Chem. **84**: 741-748, 1929.
8. Sperry, W. M., and Webb, M.: A Revision of the Schoenheimer-Sperry Method for Cholesterol Determination, J. Biol. Chem. **187**: 97-106, 1950.
9. Kunkel, H. G., Ahrens, E. H., Jr., and Eisenmenger, W. J.: Application of Turbidimetric Methods for Estimation of Gamma Globulin and Total Lipid to the Study of Patients With Liver Disease, Gastroenterology **11**: 499-507, 1948.
10. Ralli, E. P., Rubin, S. H., and Rinzler, S.: The Liver Lipids in Normal Human Livers and in Cases of Cirrhosis and Fatty Infiltration of the Liver, J. Clin. Invest. **20**: 93-97, 1941.

# THE RATE OF DISAPPEARANCE OF $Rb^{86}$ FROM THE PLASMA, THE BIOLOGIC DECAY RATES OF $Rb^{86}$, AND THE APPLICABILITY OF $Rb^{86}$ AS A TRACER OF POTASSIUM IN MAN WITH AND WITHOUT CHRONIC CONGESTIVE HEART FAILURE

G. E. Burch, M.D., S. A. Threefoot, M.D., and C. T. Ray, M.D.*
New Orleans, La.

IT IS evident that direct measurement of the rate of turnover of potassium in the body of man as well as other kaliokinetic processes requiring prolonged studies is important. Furthermore, some of these phenomena can be approached only by tracer methods. Unfortunately $K^{42}$, the radioactive isotope of potassium which is readily available, has too short a physical half-life for prolonged studies. Since rubidium and cesium have been shown to enter to some extent into the "metabolic pool" of potassium,[1-11] radiorubidium ($T_{½}$ 19.5 days) and radiocesium ($T_{½}$ 2.6 yr.) were employed in men and dogs to investigate their applicability as tracers of potassium and to study aspects of kaliokinetic processes in man with and without chronic congestive heart failure. These experiments were designed to follow the rapid and initial (first 120 minutes) rates of disappearance of $Rb^{86}$ from the plasma,† as well as the disappearance from the plasma and body as a whole over a period of approximately two months. In two experiments, $K^{42}$ and $Rb^{86}$ were injected simultaneously and their disappearance from the plasma and body and their appearance in the urine and stools were followed simultaneously as long as the physical half-life of the $K^{42}$ permitted. Not only do these studies indicate some aspects of turnover phenomena of rubidium itself, but they also present important data concerning potassium metabolism not obtainable from tracer studies with $K^{42}$. These experiments also reveal that sampling an intracellular compartment such as erythrocytes rather than sampling an extracellular compartment such as the plasma permits more accurate tracing of substances like potassium and rubidium which are distributed mainly in the intracellular compartments of the body.

## METHODS AND MATERIALS

Six adult subjects were studied, 3 of whom were control subjects without heart failure and 3 of whom were patients with chronic congestive heart failure (Table I). The subjects were studied at Charity Hospital for approximately two months under special metabolically controlled conditions. The initial 120 minutes of observation was conducted in our laboratory. A control subject was studied simultaneously under the same environmental and other experimental conditions as 2 subjects with chronic congestive heart failure. All subjects were

Aided by a Public Health Service Grant, H-143, the Mrs. E. J. Caire Fund for Research in Heart Disease, and a grant from the United States Atomic Energy Commission, AT-(40-1)-233.

Received for publication Oct. 20, 1954.

*From the Department of Medicine, Tulane University School of Medicine, and Charity Hospital of Louisiana at New Orleans, La.

†Both plasma and serum were studied and the results are applicable to either.

free from renal disease. Subject 601 was studied initially and alone for purposes of experimental orientation. Three subjects (Subjects 602, 603, and 604) were placed under carefully controlled dietary conditions,* whereas the other 3 subjects were given the ordinary hospital diet. Subjects 701 and 702 were studied for only a few days.

TABLE I. CLINICAL DATA ON THE SIX SUBJECTS STUDIED. SUBJECTS 701 AND 702 RECEIVED SIMULTANEOUSLY 50,000,000 CPM OF BOTH $K^{42}$ AND $Rb^{86}$ INTRAVENOUSLY

| SUBJECT NO. | AGE (YR.) | SEX | BODY WEIGHT (KG.) | DIAGNOSIS | DOSE | |
|---|---|---|---|---|---|---|
| | | | | | CPM | MC. |
| 601 | 34 | F | 72.8 | Tuberculous adenitis under treatment | 200,000,000 | 1.0 |
| 602 | 57 | M | 71.1 | Anxiety state | 200,000,000 | 1.0 |
| 603 | 39 | M | 55.8 | Rheumatic heart disease, moderate congestive heart failure, neurofibromatosis | 200,000,000 | 1.0 |
| 604 | 45 | M | 55.5 | Cor pulmonale, severe congestive heart failure | 200,000,000 | 1.0 |
| 701 | 51 | F | 51.2 | Rheumatic mitral insufficiency, no congestive heart failure | 50,000,000* | 0.25 |
| | | | | | 50,000,000* | 0.25 |
| 702 | 34 | F | 80.0 | Hypertension, moderate congestive heart failure | 50,000,000* | 0.25 |
| | | | | | 50,000,000* | 0.25 |

*Refers to 50,000,000 CPM $K^{42}$ and 50,000,000 CPM $Rb^{86}$.

(a) *A Study of the Rapid Rate of Disappearance of $Rb^{86}$ From the Plasma.*—For measurement of the rapid rate of disappearance of the $Rb^{86}$ from the plasma of man, the $Rb^{86}$ was injected quickly (2 to 8 seconds) into a median basilic vein while blood samples were collected as rapidly as possible (5- to 10-second intervals of time) from a median basilic vein of the opposite arm for the first 5 minutes, at 1- to 2-minute intervals for the next 5 minutes, and at increasing intervals of time until the collections were made at 10-minute intervals for the remainder of the first 120 minutes of the study. Samples of 2 ml. of blood were collected with and without heparin. The blood of the heparinized samples was centrifuged for 1 or 2 minutes immediately after withdrawal in order to separate the plasma and erythrocytes so that the rate of entrance of the $Rb^{86}$ into the erythrocytes could be measured. When the rate of flow permitted, samples of urine were collected simultaneously with each sample of blood by means of an indwelling bladder catheter.

(b) *A Study of the Slow Rate of Disappearance of $Rb^{86}$ From the Plasma and Erythrocytes.*—The subjects were kept under constant 24-hour surveillance by special nurses who controlled dietary intake and medication, measured body weight daily, collected biologic fluids and excreta, measured fluid intake, and provided other nursing services and control. A daily sample of blood was collected at approximately 9:00 A.M., and each specimen of feces and of urine was collected in a separate container. The quantity and the time each was passed were recorded. Each sample was assayed separately and then all collections for each 24-hour period were pooled and reassayed to obtain the rate of excretion per day. Any special fluids, such as vomitus, sputum, and ascitic and pleural fluids, were collected and studied. The diet was not changed except for the addition of known quantities of potassium chloride and other substances as indicated in the illustrations.

Sodium, potassium, and chloride were measured simultaneously in all body fluids studied for $Rb^{86}$. The measurements of sodium and potassium were made by means of the Beckman flame photometer[12] and the measurements of chloride by the method of Schales and Schales.[13] The method for determination of radioactivity was that previously described.[14]

For measurement of the $Rb^{86}$ content of the erythrocytes, either of two methods was employed:

1. *Direct counting:* Heparinized whole blood was centrifuged in Wintrobe hematocrit tubes and the plasma was removed for counting. After the hematocrit tubes above the

*For these studies we wish to express appreciation for the cooperation of Sister Euphemia and Mrs. Betty Doolittle of the Dietary Department of Charity Hospital.

column of red cells were rinsed twice with normal saline solution, the second rinsing fluid and upper 2 to 3 mm. of erythrocytes were aspirated. The tubes were then filled to a known level with normal saline solution and recentrifuged, the hematocrit being determined again. The cells were then thoroughly mixed in the hematocrit tube and laked by freezing in a mixture of dry ice and alcohol and then thawing. The mixture of laked red cells and normal saline solution was then counted.

2. *Indirect calculation:* This method, which was used throughout almost the entire study because it was simpler to employ and as accurate as the first method, was conducted in the following manner. An aliquot of heparinized whole blood was centrifuged in one Wintrobe hematocrit tube for the determination of hematocrit and the supernatant plasma was removed for counting as quickly as possible after collection of the sample from the patient. Another aliquot was placed in a Wintrobe hematocrit tube and laked in a mixture of dry ice and alcohol by freezing and then thawing. The radioactivity of the laked whole blood was then determined. From the hematocrit and radioactivity of the plasma and of the whole blood the content of Rb$^{86}$ in the erythrocytes was calculated.

## RESULTS

*The Early Concentration-Time Course of Rb$^{86}$ in the Blood Plasma.*—The concentration-time course of Rb$^{86}$ in the plasma for the first 120 minutes after intravenous injection of the tracer is summarized by Fig. 1. It is evident from this illustration that these concentration-time course curves are similar in general appearances to those obtained previously for Na$^{24}$, Na$^{22}$, Cl$^{36}$, and Hg$^{203}$, [15-18] but differed quantitatively. Such curves were obtained when injection of the tracer was sufficiently rapid. When the injection was relatively slow the other type of slowly ascending curve, which gradually approached a plateau as described elsewhere for Na$^{24}$ and Cl$^{36}$, [15, 17] was obtained.

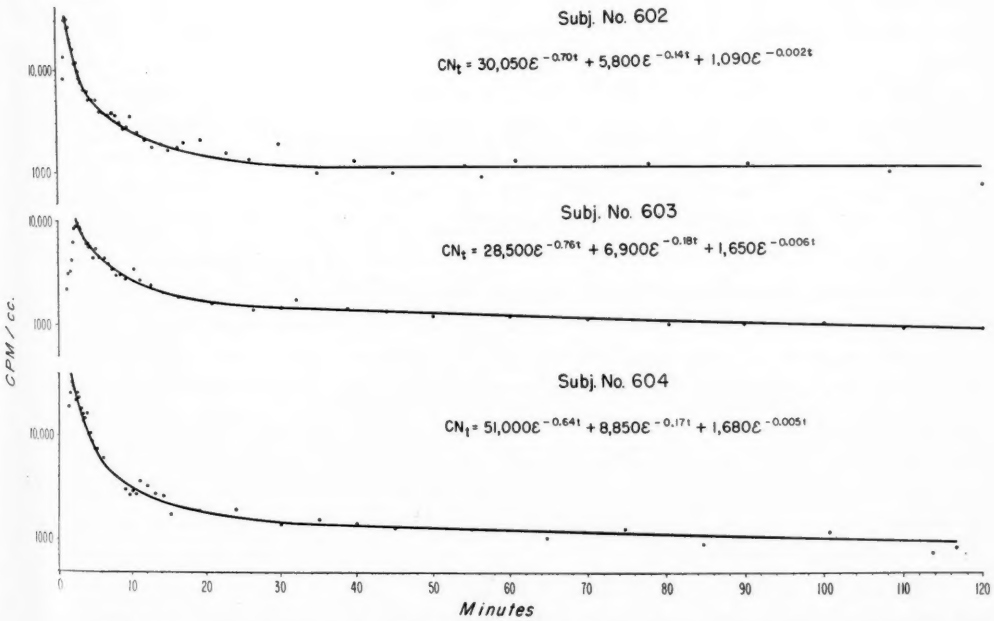



Fig. 1.—Concentration-time course of Rb$^{86}$ in the plasma of control Subject 602 and two subjects (Subjects 603 and 604) with congestive heart failure. The relative rates of exchange are indicated by the equations shown with each regression curve.

The regression curves (Fig. 1) permitted a calculation of the rate of transfer of $Rb^{86}$ from the plasma of man by the method described by Merrell and associates.[19, 20] The mathematical definition of the curves is given by the equations shown with each curve (Fig. 1). The three regression curves and their equations are very similar. Table II shows the distribution and rates of turnover of $Rb^{86}$ obtained from these concentration-time course curves for the three subjects.

TABLE II. THE DISTRIBUTION AND RATE OF TURNOVER OF $Rb^{86}$ AND $K^{42}$ BETWEEN THE PLASMA AND OTHER COMPARTMENTS

| SUBJECT | 602 | 603 | 604 | 701 | | 702 | |
|---|---|---|---|---|---|---|---|
| TRACER | $Rb^{86}$ | $Rb^{86}$ | $Rb^{86}$ | $Rb^{86}$ | $K^{42}$ | $Rb^{86}$ | $K^{42}$ |
| q = proportion of total tracer not in plasma | 0.9705 | 0.9555 | 0.9727 | 0.9437 | 0.9365 | 0.9800 | 0.9771 |
| 1 - q = proportion of total tracer in the plasma | 0.0295 | 0.0445 | 0.0273 | 0.0563 | 0.0635 | 0.0200 | 0.0229 |
| $R_s$ = proportion of plasma tracer leaving plasma per minute | 0.592 | 0.619 | 0.558 | 0.4524 | 0.545 | 0.814 | 0.952 |
| $R_e$ = proportion of nonplasma tracer returning to plasma per minute | 0.0180 | 0.0288 | 0.0157 | 0.0270 | 0.0370 | 0.0166 | 0.0223 |
| $R_s \times (1 - q) = R_e(q)$ = proportion of total body tracer moving in *or* out of the plasma per minute | 0.0175 | 0.0275 | 0.0153 | 0.0255 | 0.0347 | 0.0163 | 0.0218 |
| $R_e(q) + R_s (1 - q)$ = proportion of total body tracer moving in *and* out of the plasma per minute | 0.0350 | 0.0550 | 0.0306 | 0.0510 | 0.0694 | 0.0326 | 0.0436 |

Equations of decay curves:

Subject

602 $Rb^{86}$ $30{,}050e^{-.701t} + 5{,}800e^{-.340t} + 1{,}090e^{-.00245t}$

603 $Rb^{86}$ $28{,}500e^{-.762t} + 6{,}900e^{-.377t} + 1{,}650e^{-.00641t}$

604 $Rb^{86}$ $51{,}000e^{-.643t} + 8{,}850e^{-.370t} + 1{,}680e^{-.00480t}$

701 $Rb^{86}$ $5{,}250e^{-.592t} + 2{,}120e^{-.198t} + 440e^{-.0125t}$; $K^{42}$ $4{,}000e^{-.722t} + 1{,}500e^{-.210t} + 393e^{-.0170t}$

702 $Rb^{86}$ $14{,}700e^{-.942t} + 3{,}300e^{-.336t} + 368e^{-.00797t}$; $K^{42}$ $14{,}000e^{-1.08t} + 2{,}130e^{-.277t} + 378e^{-.00765t}$

Radiopotassium ($K^{42}$) and radiorubidium ($Rb^{86}$) were injected simultaneously from the same syringe in two subjects, one a control subject without heart failure (Subject 701) and the other with moderately severe chronic congestive heart failure (Subject 702). These subjects were studied as described for the other $Rb^{86}$ tracer experiments until the physical decay of the $K^{42}$ made it impossible to measure the $K^{42}$ accurately. This experiment was conducted to investigate the accuracy with which $Rb^{86}$ traced the rate of exchange of potassium between the plasma and extraplasma compartments of man in vivo.

The results of the concentration-time course curves for the first 120 minutes after injection of the two tracers are summarized in Fig. 2. It is evident from the curves that $Rb^{86}$ and $K^{42}$ behaved in a similar fashion. In fact, the curves for the two isotopes for both subjects can almost be superimposed. The equations defining both sets of curves for each subject differed relatively little, the differences being due largely to the method of obtaining the equations from the plots.

By means of the methods described by Merrell and associates,[19, 20] the rate of turnover and the plasma and extraplasma distribution of both the $Rb^{86}$ and $K^{42}$ were calculated (Table II) from the concentration-time course curves and

their respective equations. It is again evident, as would be expected from the similarity of concentration-time course curves, that the plasma and extra-plasma distribution and rates of turnover of $Rb^{86}$ and $K^{42}$ were very similar.

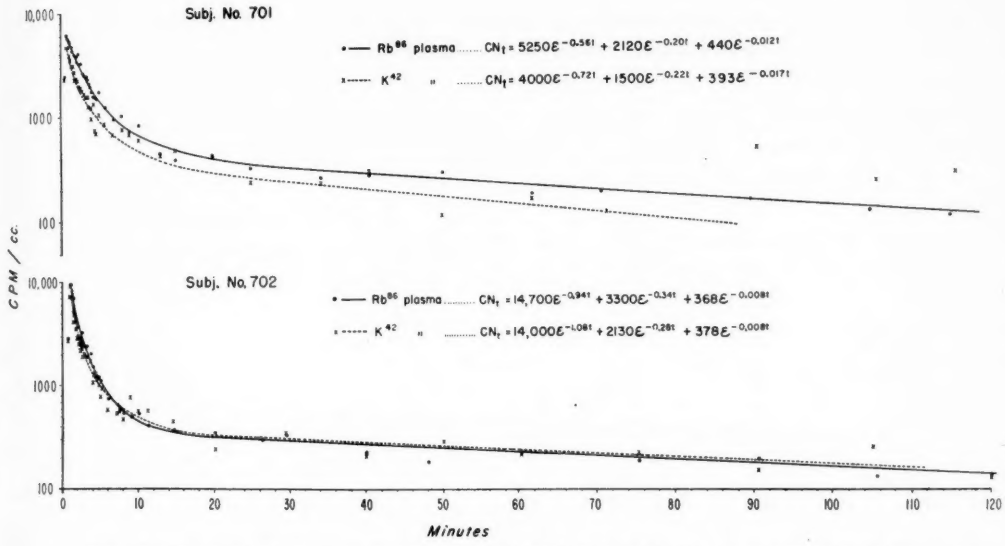



Fig. 2.—Concentration-time course of $Rb^{86}$ and $K^{42}$ injected simultaneously into control Subject 701 and Subject 702 with congestive heart failure. The rates of regression are expressed by the equations. There is a striking similarity in the concentration-time course of $K^{42}$ and $Rb^{86}$ in each of the subjects.

*Comment.*—During the first 120 minutes the rate with which $Rb^{86}$ and $K^{42}$ disappeared from the plasma was slower than that found for $Cl^{36}$ (Reference 17), about the same rate as noted previously for $Hg^{203}$ (Reference 18), and slightly faster than that for $Na^{24}$ and $Na^{22}$ (References 15 and 16). There were considerable individual variations in the curves in all groups of subjects studied with the various isotopes. It is not possible to know if these differences were related to their respective clinical states, body size, rates of mechanical mixing throughout the body fluids, rates of diffusion, or other physiologic factors. The various problems involved in the interpretation of such concentration-time course curves have been discussed elsewhere[18] and require no further comment for $Rb^{86}$. Mechanical mixing in the circulation and the rate of injection remain important factors which determine the shape of such curves.

The similarity of the concentration-time course for $Rb^{86}$ and $K^{42}$ indicates the similarity of the various physiologic factors responsible for the regression in concentration of these tracers during the first 120 minutes following the injection. Although the regression curves for both $Rb^{86}$ and $K^{42}$ were the same in the individual subjects, there were differences between the curves of the two subjects studied.

That $Rb^{86}$, with twice the mass of $K^{42}$, is able to move with about the same rate in biologic fluids is an interesting physicochemical fact. It may be related to the fact that, in spite of its atomic weight, the $Rb^{86}$ ion in the

hydrated state is approximately the same size as the $K^{42}$ ion in its hydrated state.[21] There must be other important physical and physiologic factors as well which resulted in the similarity of the concentration-time course curves for $Rb^{86}$ and $K^{42}$ for the first 120 minutes.

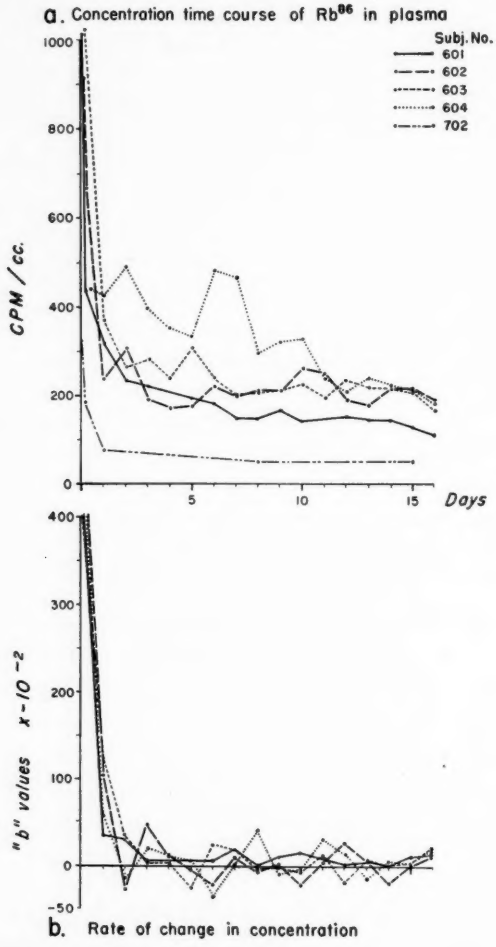



Fig. 3.—*A*, Concentration-time course of $Rb^{86}$ in the plasma of five subjects for the first sixteen days of observation. *B*, Rate of change in the plasma concentration of $Rb^{86}$. The ordinate values represent the values of the exponent "b," an expression of the slope of the concentration-time course curves.

*Equilibrium of Distribution.*—Radiorubidium has a half-life sufficiently long to permit a study of the time at which equilibrium of distribution becomes established throughout the body. As indicated in a previous study with $Cl^{36}$ (Reference 22), it was not possible to measure the concentration of the tracer in all of the compartments of the body in order to determine when specific activity becomes equal and stable throughout the body. It, therefore, was necessary that the time at which equilibrium of distribution is established be determined indirectly. If a steady state exists and intake and output of the

nontracer are constant, the concentration-time course curve of the sampled compartment (blood plasma) will develop a constant slope. This constant slope is readily apparent from the "b" values[17] of the curves. In a previous publication[22] it was stated that equilibrium of distribution could not exist until a steady state of exchange between compartments existed. It was suggested that when the "b" values for the concentration-time course curves of the tracers of sodium and chloride became constant in the serum, equilibrium of distribution of the tracers tended to exist, but certain limitations of this type of analysis were indicated. However, it is evident from these experiments with Rb$^{86}$ that such an approach can be erroneous, particularly if the plasma compartment alone is followed, and that this type of analysis was applicable in previous studies because the sampled compartment (plasma)

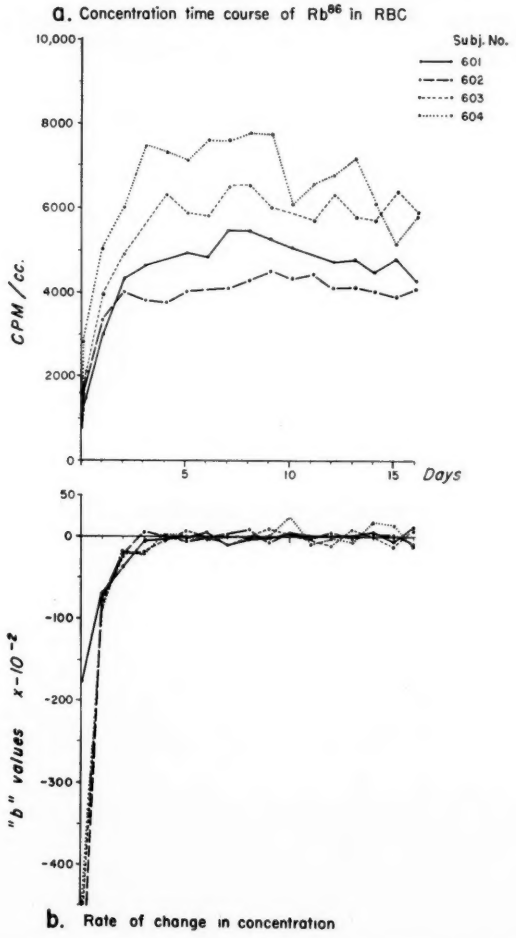



Fig. 4.—*A*, Concentration-time course of Rb$^{86}$ in the erythrocytes for the first sixteen days of observation. The values shown in this graph were calculated from the concentration of Rb$^{86}$ in the plasma and whole blood and the hematocrit. The concentration of Rb$^{86}$ continues to rise until the fifth to eighth day. *B*, Rate of change in the concentration of Rb$^{86}$ in the erythrocyte. The ordinate values are those of the exponent "b."

contained a large mass of the tracer and nontracer and any compartments which exchanged slowly were relatively small or the rate was extremely slow, but errors did exist.

The "b" values and concentration-time course curves which represent rates of decrease or increase of $Rb^{86}$ in the various compartments studied are summarized in Figs. 3 to 9. These curves show a tendency for the plasma, erythrocytes, and whole blood compartments to reach a constant rate of change in $Rb^{86}$ concentration not earlier than five days. It is evident from inspection of these figures and Table III that the concentration-time course for the $Rb^{86}$ in the plasma declined more rapidly than that for urinary or total excretion of $Rb^{86}$. Therefore, equilibrium of distribution could not have existed as long as this discrepancy was present. Even if these curves had declined at the same rate, equilibrium of distribution would not necessarily have existed anyway. An attempt to estimate from these curves when equilibrium of distribution could possibly have occurred was unsuccessful.

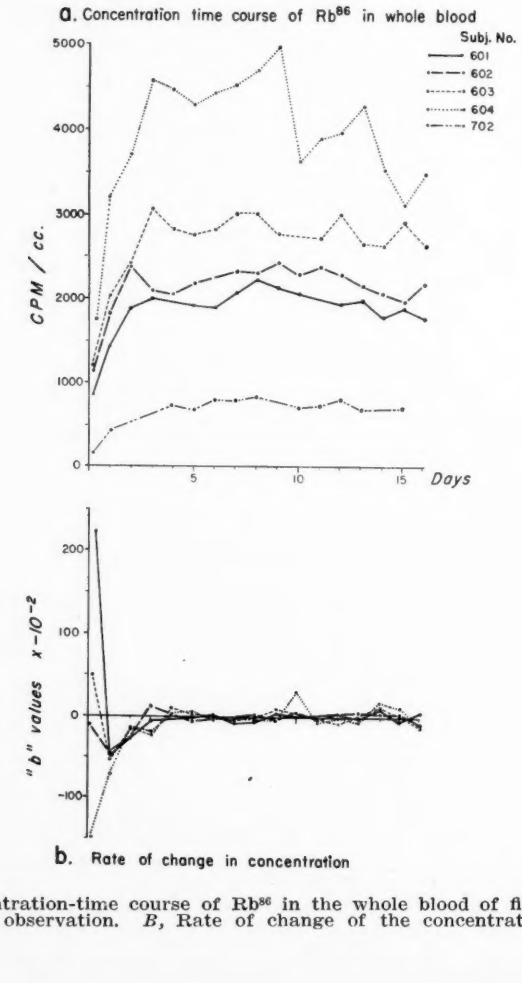



Fig. 5.—*A*, Concentration-time course of $Rb^{86}$ in the whole blood of five subjects for the initial sixteen days of observation. *B*, Rate of change of the concentration of $Rb^{86}$ in the whole blood.

*Comment.*—From the concentration-time course curves of $Rb^{86}$ in the plasma, erythrocytes, and whole blood as well as the "b" values for these curves, it is not possible to estimate the time at which equilibrium of distribution of the tracer occurred. As indicated previously, when the "b" values become constant and the rate of regression of these curves constant and equal, equilibrium of distribution of the tracer does not necessarily exist. That this is true is evidenced by the fact that the regression curves of excretion and their "b" values as well as the regression curves of the plasma and their "b"

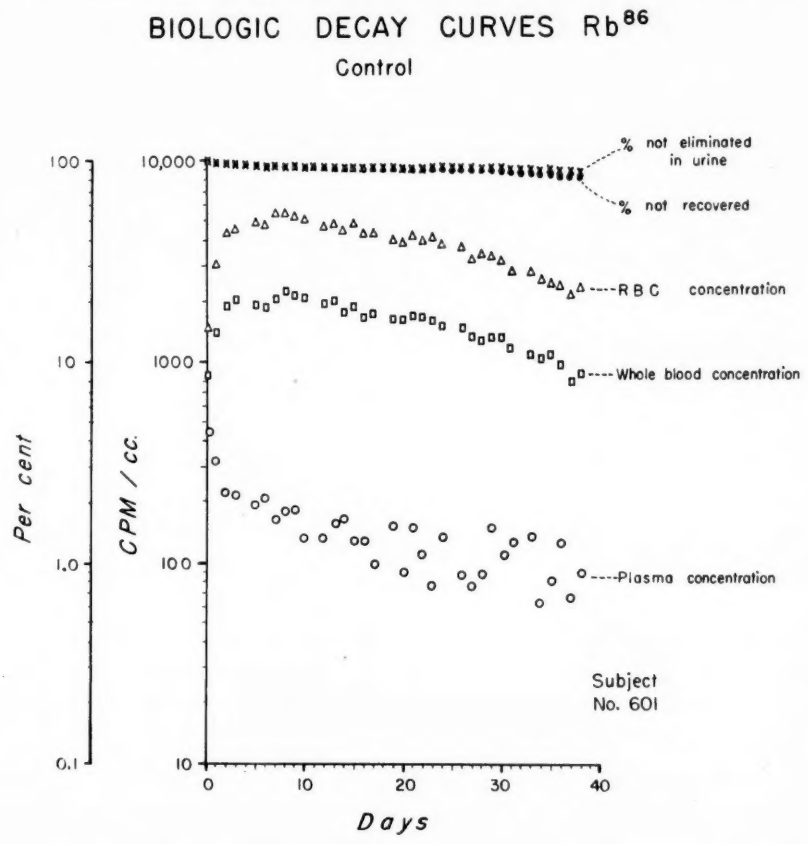



Fig. 6.—Concentration-time course of $Rb^{86}$ in the plasma, erythrocytes, and whole blood and the time course of urinary excretion and total excretion of $Rb^{86}$ in control Subject 601. Urinary and total excretion are expressed as the percentage of the administered dose of tracer remaining in the body and not eliminated by these routes of excretion.

values continued to be different when the concentration-time course curves for the plasma and erythrocytes had reached a constant slope. The rate of decline in concentration of $Rb^{86}$ in the plasma was greater than the rate of excretion of $Rb^{86}$ from the body. For this to occur, equilibrium of distribution must not have existed between the plasma and extraplasma compartments of the body.

Because the serum or plasma compartment for $Rb^{86}$ is a small one and the other compartments exchanging with the plasma compartment are relatively

large and the rates of exchange relatively slow, and because of difficulties indicated in the estimation of equilibrium of distribution by indirect methods other than the measurement of specific activity in all the compartments of the body, it was not possible to determine in these studies when equilibrium of distribution was reached. In fact, it can be stated that it was not reached at any time in these studies extending for as long as four to six weeks. That

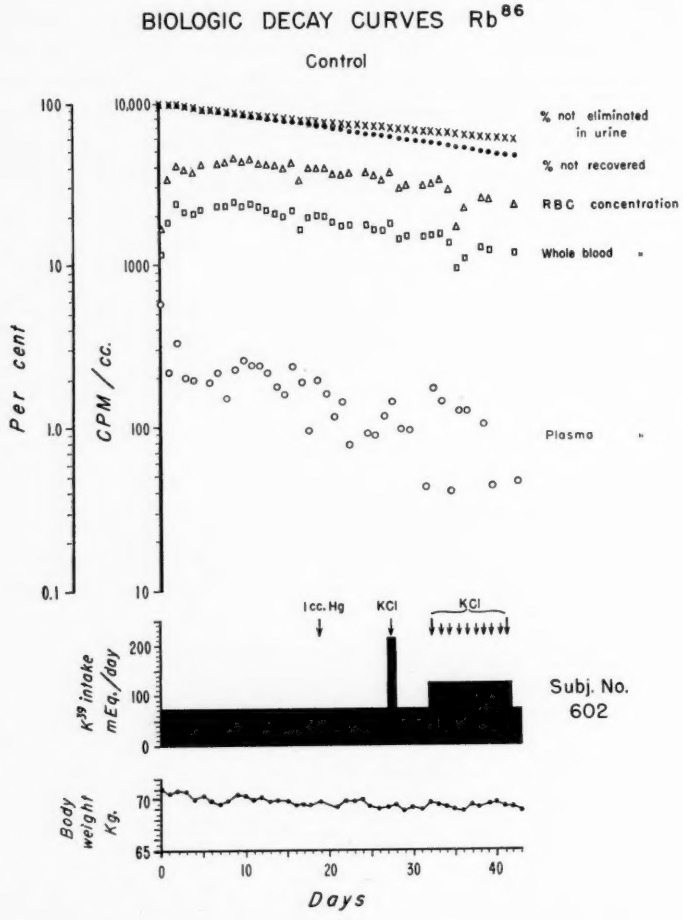



Fig. 7.—Concentration-time course of $Rb^{86}$ in the plasma, erythrocytes, and whole blood and the time course of total and urinary excretion of $Rb^{86}$ in control Subject 602. The relationship to dietary potassium intake, drug administration, and body weight is shown.

equilibrium of distribution was not reached in the first five or more days is indicated by Figs. 4 and 5, which showed a progressive continuous rise of the concentration of $Rb^{86}$ in the erythrocytes. It is quite likely that other compartments of the body, such as brain and bone, exchange $Rb^{86}$ with the plasma more slowly than the erythrocytes.[23] It should be noted that the intake and output of rubidium were not measured, but the intake of potassium which

TABLE IIIA. BIOLOGIC DECAY RATES OF $Rb^{86}$ DETERMINED BY TWO METHODS: (1) INSPECTION OF SLOPES OF DECAY CURVES, AND (2) MATHEMATICAL DETERMINATION FROM CALCULATED SLOPES ("B" VALUES)

| | FROM CURVES | | | | | CALCULATED FROM "B" VALUES (EXCLUDING FIRST 5 DAYS) | | |
|---|---|---|---|---|---|---|---|---|
| | $C_{½}$ (DAYS) | | | | | | | |
| SUBJECT | PLASMA | RBC | WHOLE BLOOD | $U_{½}$ (DAYS) | $E_{½}$ (DAYS) | $C_{½}$ PLASMA (DAYS) | $U_{½}$ (DAYS) | $E_{½}$ (DAYS) |
| 601* | 29.0 | 26.5 | 25.5 | 168.0 | 124.0 | 36.5 | 188.4 | 133.9 |
| 602 | 16.5 | 42.0 | 33.0 | 55.0 | 38.5 | 9.8 | 58.9 | 39.2 |
| 603 | 23.5 | 34.0 | 31.5 | 84.5 | 53.0 | 18.6 | 85.8 | 57.0 |
| 604 | 57.0 | 38.5 | 43.0 | 127.0 | 37.5 | 35.0 | 140.0 | 42.8 |

*Subject 601 was not on a known potassium diet and therefore cannot be compared with the other subjects.

TABLE IIIB. THE INFLUENCE OF POTASSIUM UPON THE BIOLOGIC DECAY RATES

| SUBJECT | PARAMETER | MEAN, ENTIRE STUDY EXCLUDING FIRST 5 DAYS | HIGH K INTAKE | LOW K INTAKE |
|---|---|---|---|---|
| | | *Intake 92.10 meq./day* | *Intake 126.71 meq./day* | *Intake 74.39 meq./day* |
| 602 | $C_{½}$ | 9.8 | 5.2 | 8.3 |
| | $U_{½}$ | 58.9 | 56.7 | 59.8 |
| | $E_{½}$ | 39.2 | 35.4 | 41.3 |
| | | *Intake 80.68 meq./day* | *Intake 110.39 meq./day* | *Intake 64.99 meq./day* |
| 603 | $C_{½}$ | 18.6 | 12.8 | 18.2 |
| | $U_{½}$ | 85.8 | 89.9 | 84.8 |
| | $E_{½}$ | 57.0 | 70.6 | 53.6 |
| | | *Intake 66.81 meq./day* | *Intake 95.66 meq./day* | *Intake 56.56 meq./day* |
| 604 | $C_{½}$ | 35.0 | 16.8 | 18.4 |
| | $U_{½}$ | 140.0 | 278.0 | 151.3 |
| | $E_{½}$ | 42.8 | 69.1 | 41.0 |

It should be noted that $C_{½}$ was shortened by the high intake. In the entire study and on the various diets, $C_{½}$ and $U_{½}$ were greatest for the subject with severe CHF and least for the control subject. Since Subject 604 had a severe diarrhea with much potassium loss, the $E_{½}$ for this subject was considerably shorter than $U_{½}$.

influences rubidium turnover was measured and controlled. Because $Rb^{86}$ seems to enter the "metabolic pool" of potassium, these data would apply to equilibrium of distribution of potassium as well. Furthermore, these concentration-time course curves seem to support the fact that sampling of the erythrocytic compartment, an intracellular compartment, reflects more accurately the rubidiokinetic and kaliokinetic processes than sampling the plasma, an extracellular compartment.

*Biologic Decay Rates.*—$C_{½}$, $U_{½}$, and $E_{½}$ values were measured and the results are summarized in Table III and in Figs. 6 through 14. Because the concentration-time course of $Rb^{86}$ was observed for the erythrocytes and for the whole blood as well as for the plasma, rates of decay were calculated for all three of these. These parameters have been defined and described in detail elsewhere.[17, 24, 25]

The average values of the parameters were obtained by fitting a curve to the concentration-time course curves of $Rb^{86}$ in the plasma, red blood cells, and whole blood and from the time course curves of the percentage of $Rb^{86}$ remaining in the body after measuring urinary excretion and after measuring

other routes of excretion, including the sum total of the urine, feces, sputum, and vomitus. The equation for determining the percentage of $Rb^{86}$ remaining is:

$$\%N_t = 1 - \frac{\sum_{s=1}^{t} k_s}{N_o} \times 100. \qquad (1)$$

The results obtained by this method are shown in Table III.

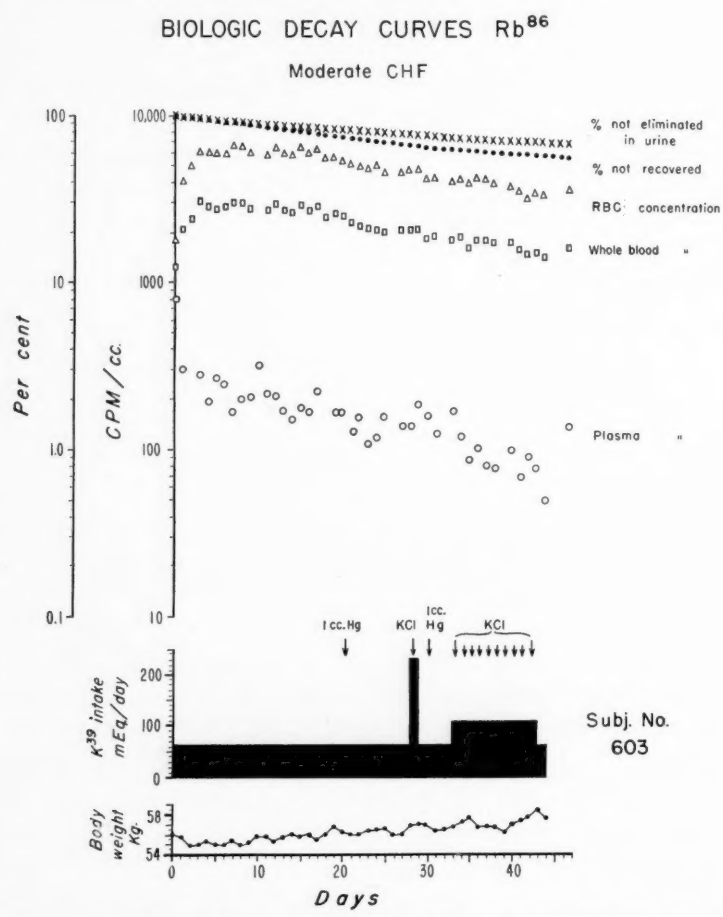



**Fig. 8.**—Concentration-time course of $Rb^{86}$ in the plasma, erythrocytes, and whole blood and the time course of urinary and total excretion of $Rb^{86}$ in Subject 603 with moderate congestive heart failure.

The calculation of $C_{½}$, $U_{½}$, and $E_{½}$ by drawing a mean regression line by inspection among the observed points is subject to variations. Because of possible errors, these parameters were also calculated by obtaining the averages of the daily slopes (the value of "b") of the respective regression curves. The procedure for calculating the "b" values has been presented elsewhere.[17]

*Biologic Decay Rates Determined by Means of Study of "b" Values.*—The $C_{½}$, $U_{½}$, and $E_{½}$ values obtained from the averages of the "b" values are shown

in Table III. These are more accurate than those obtained from the drawing of mean regression curves among the observed respective values. Both sets of values are presented for purposes of comparison.

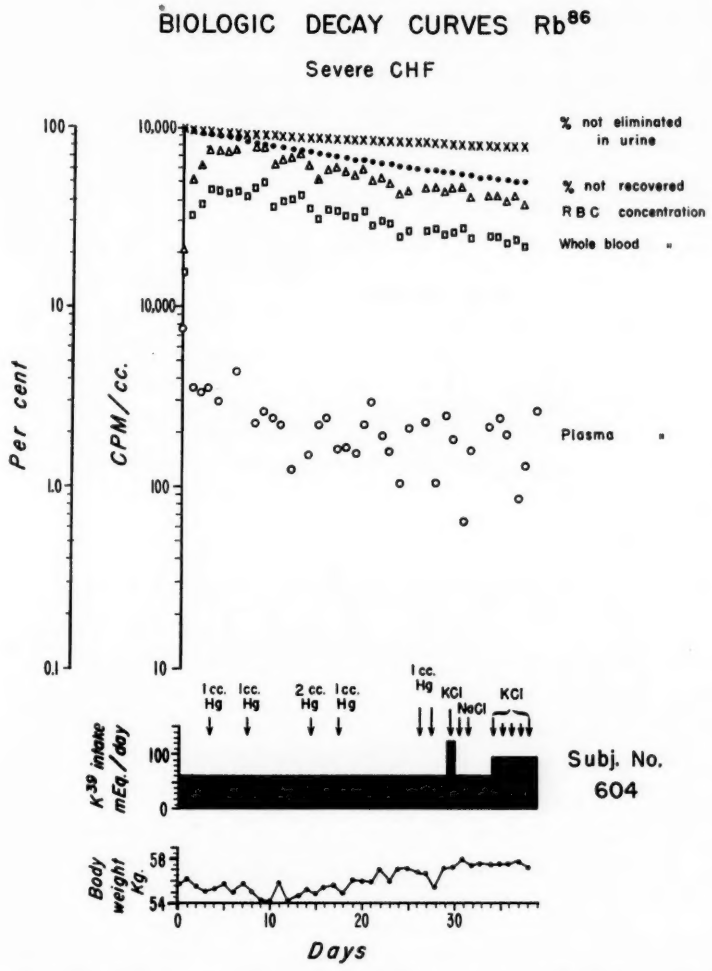



Fig. 9.—Concentration-time course of Rb$^{86}$ in the plasma, erythrocytes, and whole blood and the time course of urinary and total excretion of Rb$^{86}$ in Subject 604 with severe congestive heart failure.

It is evident from Table III that for the control subjects without congestive heart failure from 9.8 to 36.5 days were required for the Rb$^{86}$ to reach one-half the concentration in the plasma, the time being measured after the fifth day following injection of the tracer. The first five days were excluded to allow for equilibrium of distribution to develop sufficiently for satisfactory measurement of the biologic decay rates. From 58.9 to 188.4 days were required for urinary excretion of one-half of the Rb$^{86}$ remaining in the body on the fifth day following the injection of the tracer and 39.2 to 133.9 days for combined urinary and fecal excretion in the four subjects.

Figs. 6 through 13 show the large variations in the respective biologic decay rates. The variations in "b" values were larger in absolute numbers for the plasma than for the urine and feces. At times the rates of biologic decay were influenced by dietary intake of potassium. Mercurial diuretics had no detectable effects upon the "b" values (Figs. 10 through 13). The "b" values for the control subjects and those with chronic congestive heart failure were not significantly different. It is evident that the plots of the "b" values for plasma did not always vary in the same direction or magnitude as the whole blood or erythrocytes; the latter two varied concordantly.

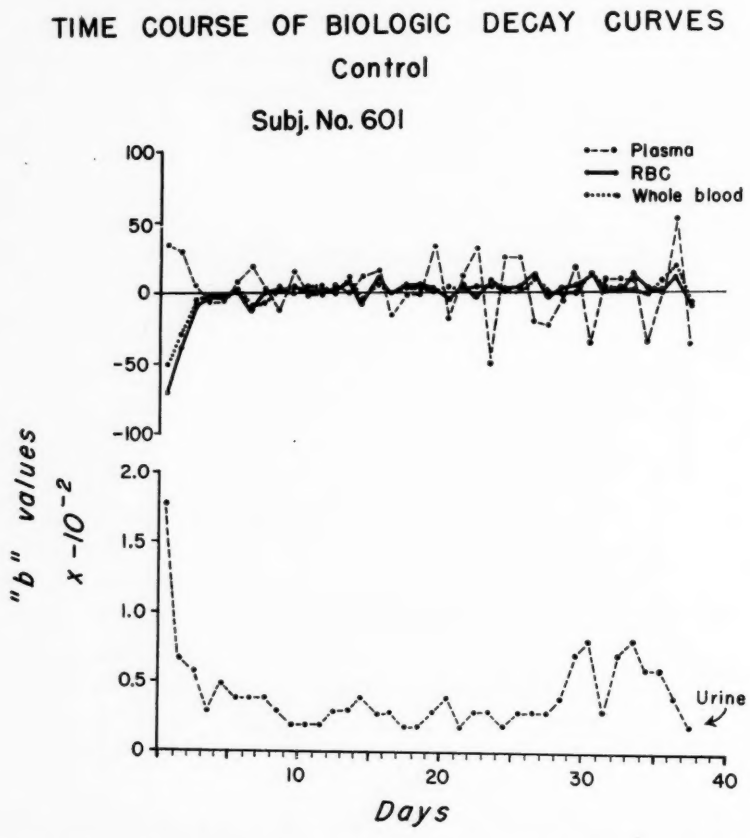



Fig. 10.—Daily variations in the slopes, expressed as "b" values, of the concentration-time course curves of plasma, erythrocytes, and whole blood and of urinary excretion of $Rb^{86}$ in control Subject 601. The values above the zero ordinate level are negative and those below are positive. Because of low values the urinary excretion is presented separately.

*Comment.*—Because there were such marked variations in the "b" values of the regression curves, it becomes evident that data obtained for a short interval of time would produce inaccurate impressions of the rate of excretion of $Rb^{86}$ or the rate of disappearance from the blood plasma, whole blood, or erythrocytes. However, it was possible from many serial observations to define the *average* behavior fairly accurately. Neither the average of many observations nor any single observation presents a complete description of the

changes, but many serial observations plotted for a fairly long period of time define the physiologic state more clearly. If Rb$^{86}$ traces potassium adequately to justify some conclusions concerning kaliokinetic phenomena, it becomes evident from the plots of the time course of the "b" values, $C_b$, $U_b$, and $E_b$ curves, that, because of the short physical half-life of $K^{42}$, there are many important physiologic aspects of potassium metabolism which $K^{42}$ could not reflect. These Rb$^{86}$ experiments exhibit the same problems indicated in studies of Cl$^{36}$ space and mass.[26-28]

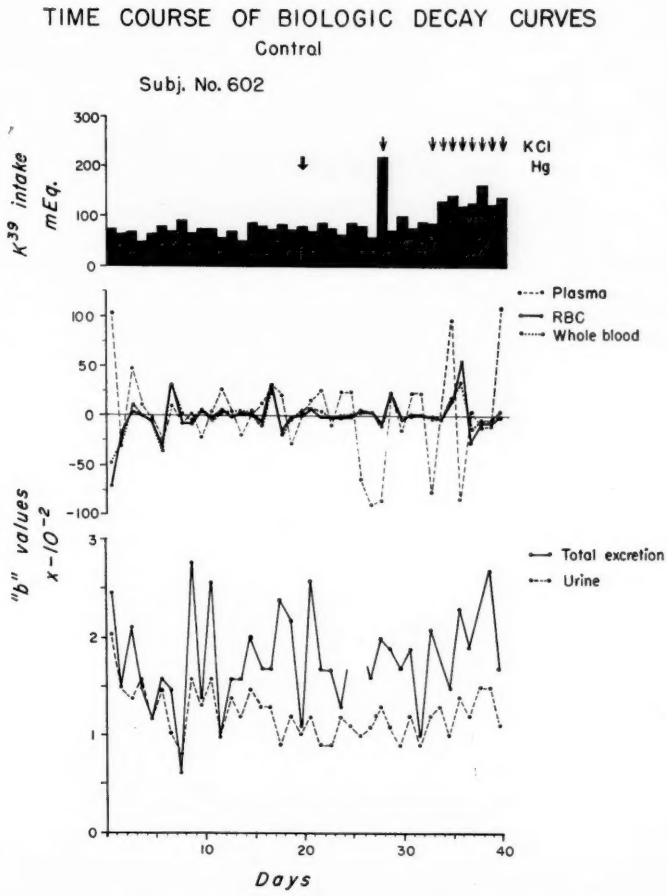



Fig. 11.—Data for control Subject 602. Method of presentation is the same as for Fig. 10. Dietary intake of $K^{39}$, drugs administered, and total excretion are shown in this graph.

The urinary and fecal excretion of Rb$^{86}$ varied considerably, thus showing further the importance of obtaining many successive observations to describe adequately the urinary and fecal elimination of Rb$^{86}$. Although continuous observations over prolonged periods of time reduce the number of subjects that can be studied in a given time, as stated previously they present a more valid description of the physiologic state.

*Rates of Biologic Decay of $Rb^{86}$ Obtained Directly From Concentration-Time Course Curves.*—The data to follow indicate observations of the rates of biologic decay of $Rb^{86}$. *If $Rb^{86}$ traces kaliokinetic phenomena satisfactorily,* the data describe the rates of biologic decay of potassium in general terms.

The results of the measurements of the rates of biologic decay obtained by drawing mean slopes through the regression curves shown in Figs. 6, 7, 8, and 9 are summarized in Table III.

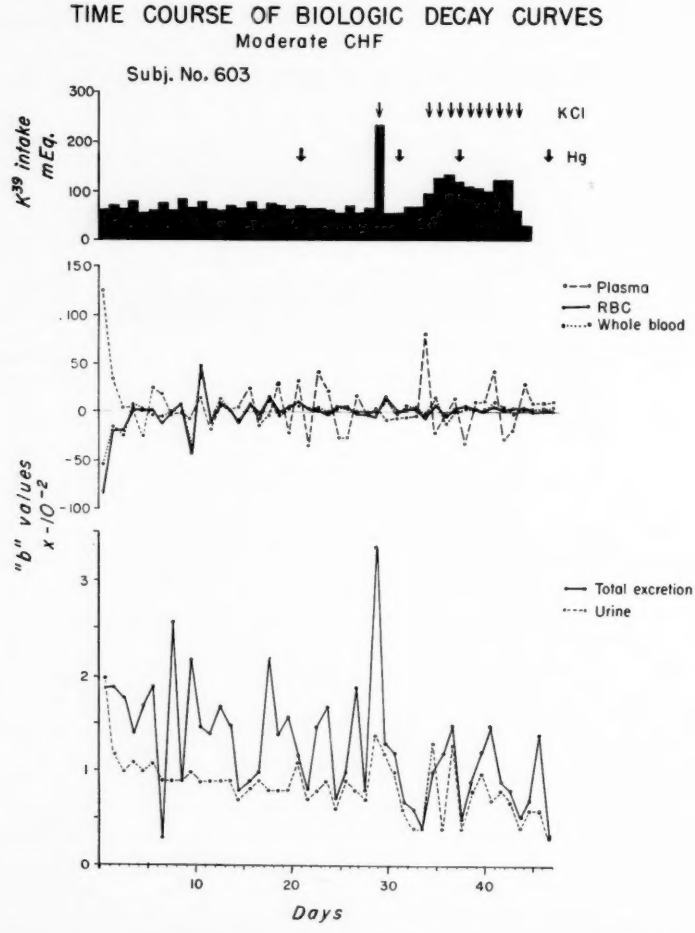



Fig. 12.—Data for Subject 603 with moderate congestive heart failure. Method of presentation of the data is the same as in Figs. 10 and 11. (Consult text for details.)

It is evident from Table III that there are considerable variations in the rate with which $Rb^{86}$ is turned over in the body. At least one-half of the $Rb^{86}$ injected was eliminated in from 37.5 to 124 days. The data obtained under the conditions of these experiments failed to reveal any demonstrable influence of relatively high and low intakes of potassium upon the biologic decay rates of $Rb^{86}$, except for $C_{1/2}$ (Table IIIB). The quantity of potassium in the high potassium diet was almost twice that in the low potassium diet.

*Comment.*—Although these observations of the rates of biologic decay were for Rb$^{86}$ itself, it will be *assumed* that the Rb$^{86}$ traced potassium closely enough to permit a discussion of rates of potassium turnover as well as that of Rb$^{86}$.

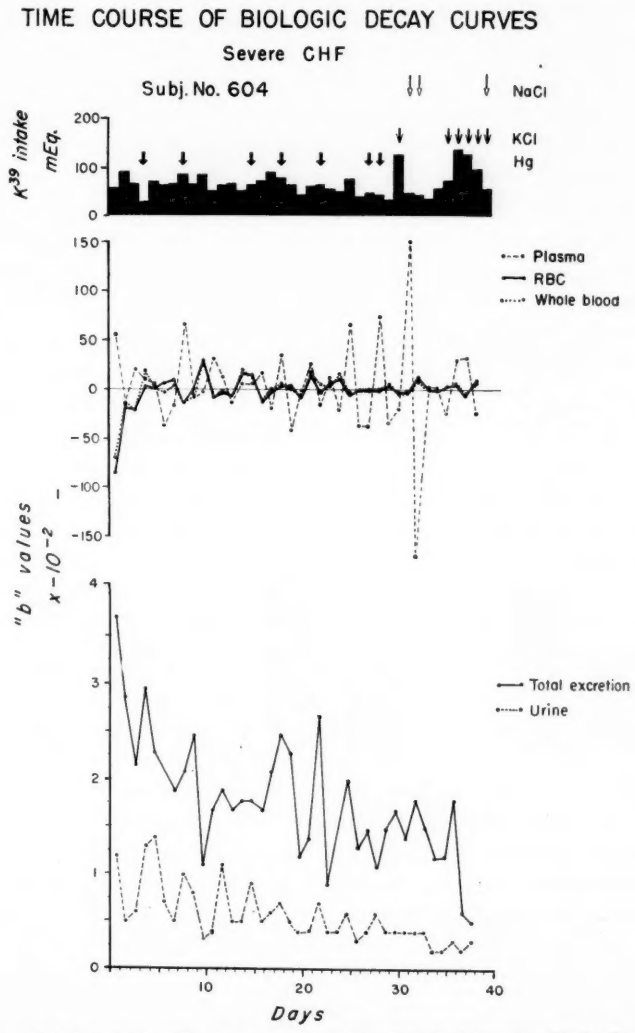



Fig. 13.—Data for Subject 604 with severe congestive heart failure. Method of presentation of the data is the same as in Figs. 10 and 11. (Consult text for details.)

The rate of turnover of Rb$^{86}$ was definitely slower than that previously observed for Na$^{22, 25}$ and Cl$^{36}$ (Reference 18). It is evident that the rate of decline in concentration of Rb$^{86}$ in the plasma exceeded that for the erythrocytes and the percentage of the injected dose remaining in the body. The latter two tended to be more alike. Thus, studying an extracellular compartment such as the plasma when attempting to observe the kinesis of primarily intracellular substances like rubidium and potassium can be inadequate if the major portion of the mass and space of the substance traced are under study.

It is all right to sample the minor compartment if the data are interpreted cautiously and especially if the major compartment is also observed. When the kinetic phenomena of sodium and chloride were studied previously, it was then advisable to observe the extracellular compartment, the plasma or serum, following the intracellular ones as the experiments dictated. To have focused upon the intracellular compartment without observing an extracellular one would have offered considerable difficulties in those observations. However, in the observations presented here the data for the erythrocytes reflect more accurately the rubidiokinetic and kaliokinetic phenomena for the body as a whole than did the study of the plasma. For example, the values for the plasma in Table IIIA indicate that control Subject 602 had a more rapid rate of turnover of $Rb^{86}$ than did the subjects with congestive heart failure. However, the values for the erythrocytes and total excretion, which are in fairly close agreement with each other, do not confirm this. The other control subject (Subject 601) could not be used in these comparisons because her dietary intake was not carefully controlled. When urinary excretion was markedly depressed during phases of severe failure and when this depression lasted for prolonged periods of time without associated diarrhea and vomiting, the biologic decay rate of potassium slowed. Large intakes of potassium for sufficiently long periods of time would be expected to increase the rate of biologic decay for reasons discussed elsewhere.[17] The changes in dietary intake of potassium changed the $C_{½}$ values of $Rb^{86}$, but not those of $U_{½}$ and $E_{½}$ (Table IIIB). The $U_{½}$ and $E_{½}$ values were not changed significantly, in part because of the relatively slow rates of exchange between the intra- and extracellular compartments and the "damping" influence of the large mass of extraplasma potassium. If the various levels of dietary intake of potassium had been maintained for long periods of time, changes probably would have been noted. Unfortunately when it was considered appropriate for these experiments to change the diet, the $Rb^{86}$ counts were near background level, thus making it impossible to make sufficiently accurate measurements. The greater variations in values shown in Figs. 6 through 13 near the final few days of the experiments are reflections, in large part, of the greater errors in counting due to levels of counts near background.

## GENERAL DISCUSSION

It is important to realize that $Rb^{86}$ was the substance actually traced in these studies. The values obtained for the various turnover phenomena and other physiologic processes certainly apply to rubidium in the quantities existing in the body. Because of previous physiologic, pharmacologic, and biochemical knowledge, as well as data obtained in this laboratory during the past two years with observations on $Rb^{86}$, $K^{42}$, and $K^{39}$, it is safe to draw general impressions and conclusions of importance concerning kaliokinetic processes that would be impossible from the use of $K^{42}$ as a tracer. However, interpretations and applications of these impressions of kaliokinetic phenomena must be made with realization that an isotope of potassium was not used. For more precise applications, crucial experiments must be checked with an isotope of potassium.

It is of interest that $Rb^{86}$, with essentially twice the mass of $K^{39}$, could serve as a useful tracer of potassium. It, of course, is not a new observation. Deuterium, $H^2$, with a mass twice that of ordinary hydrogen, $H^1$, has been of considerable value in tracer studies of hydrogen. That deuterium is different from hydrogen in biologic processes is evident from its toxic actions alone.

The observation that $Rb^{86}$ and $K^{42}$ behaved similarly immediately (for the first two hours, at least) after the simultaneous injection of the two is in agreement with studies in vitro of the rates of exchange between plasma and erythrocytes in which the two isotopes did not manifest distinguishable differences. These findings do not necessarily indicate that $Rb^{86}$ and $K^{42}$ would behave similarly for longer periods of time.. By measuring the urinary excretion of $Rb^{86}$ and $K^{39}$ during the entire period of study, it was found that although the clearance of the two substances varied concordantly it differed quantitatively.[29] This indicates a difference in the excretory patterns and kinesis of $Rb^{86}$ and $K^{39}$, although the two substances behaved remarkably alike.

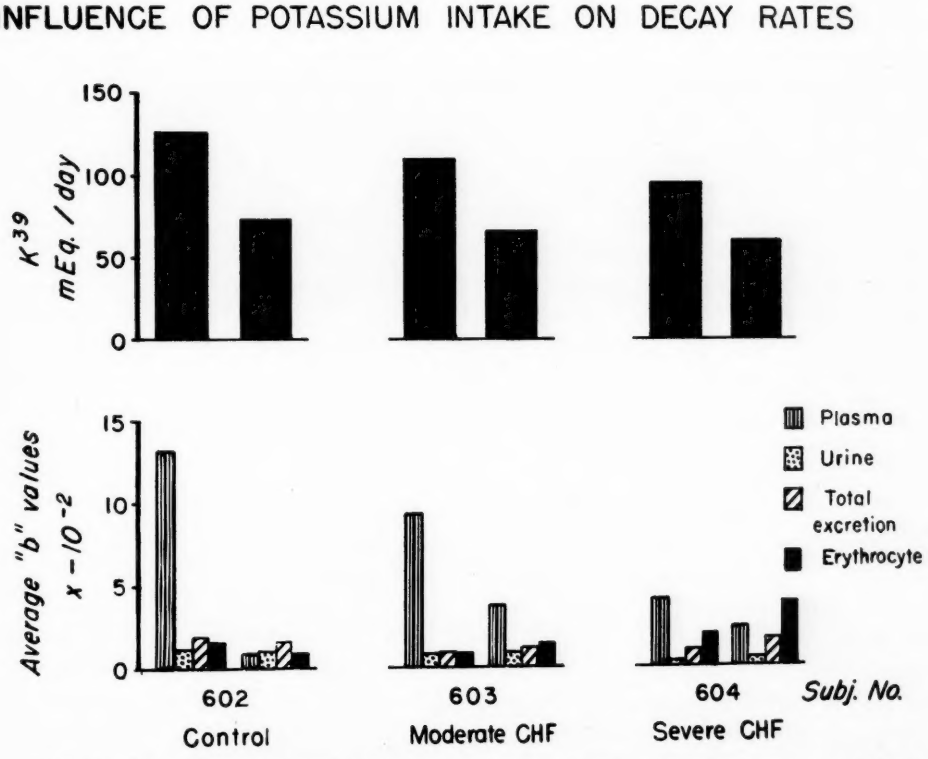



Fig. 14.—The effect of dietary intake on the rate of decline in concentration of $Rb^{86}$ in plasma and urinary and total excretion. The "b" values shown here are mean "b" values for several days on high and low potassium intake. The larger "b" value for the plasma indicates a more rapid decline in concentration of $Rb^{86}$ while on a high intake than while on a low intake of potassium. The urinary and total excretions were little affected by dietary intake.

The experiments with $Rb^{86}$ indicated a rapid exchange of $Rb^{86}$ and of potassium of the plasma with some compartments in contact with the plasma. Other compartments such as the erythrocytes exchanged slowly. Bone and

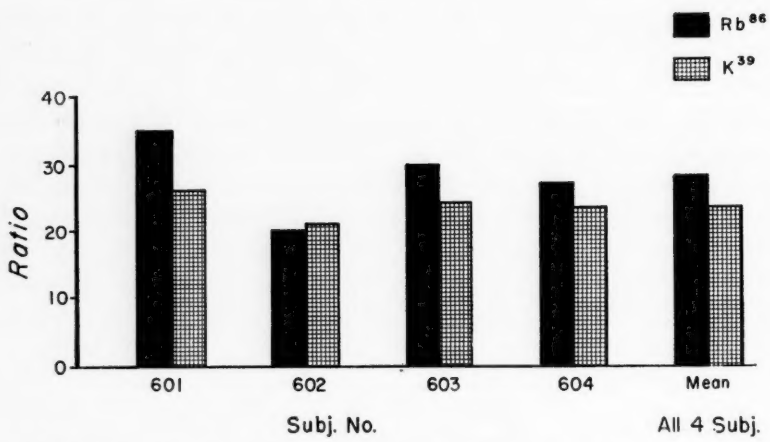



Fig. 15.—The ratio of concentration of $Rb^{86}$ and $K^{39}$ in the erythrocytes to that in the plasma. In control Subject 602 the ratios were almost equal whereas in all other subjects the erythrocyte-plasma ratio of $Rb^{86}$ was greater than the ratio of $K^{39}$.

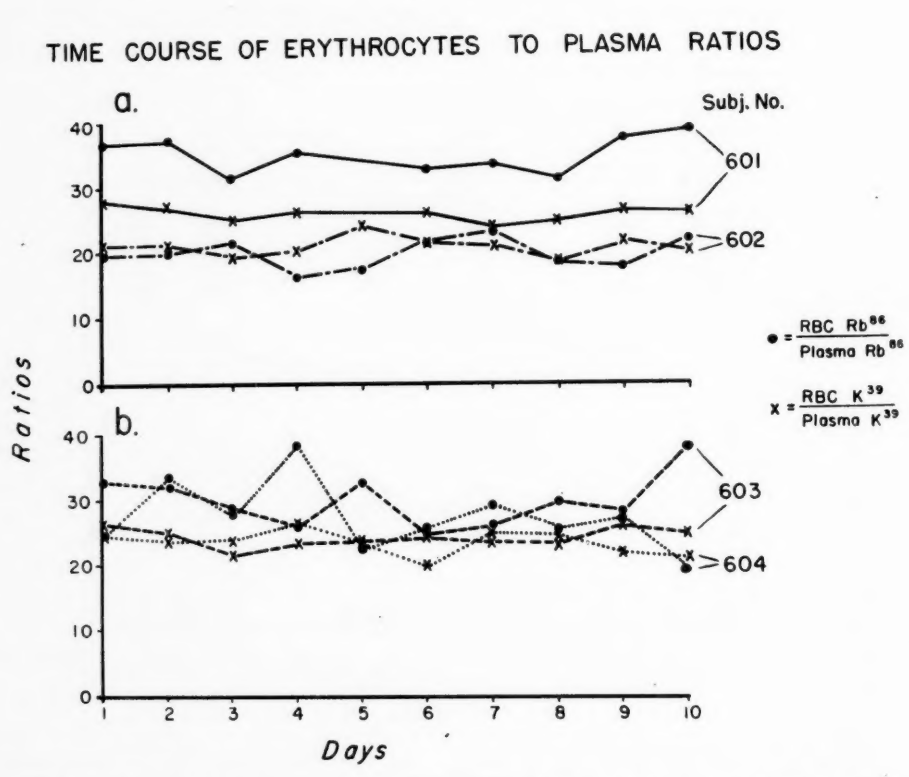



Fig. 16.—*A*, Daily variations in the concentration of $Rb^{86}$ and potassium in the erythrocytes to that in the plasma for two control subjects (Subjects 601 and 602). *B*, Same parameters for the two subjects with congestive heart failure.

the central nervous system exchanged even slower than erythrocytes.[23] Because of the failure of $Rb^{86}$ to reach equilibrium of distribution, surely $K^{42}$ is not suitable for the accurate measurement of potassium space and mass although it has been used for such purposes.[30, 31]

The potassium compartments of the body apparently fluctuate in their size and their rates of exchange among themselves, and the concentration of potassium in the intra- and extracellular fluids does not remain at a constant level. The rate of turnover for the body as a whole was definitely less than that observed for sodium and chloride with $Na^{22}$ and $Cl^{36}$, probably because of the large sluggishly exchanging intracellular mass of potassium in the body. Since the total amount of potassium in the extracellular fluid is relatively small, the amount ingested per day represents a large percentage of the extracellular potassium so that extracellular potassium exchanged more rapidly with sudden increases in potassium intake or excretion than the intracellular potassium as indicated by the values of biologic decay (Table III). This is exemplified by Figs. 8 and 14, which show a rapid decline in the concentration of $Rb^{86}$ in the plasma without an associated change in the concentration of $Rb^{86}$ in the erythrocytes or whole blood. It would appear that the high intake of potassium "washed" the $Rb^{86}$ and potassium out of the plasma into the urine in the maintenance of balance between intake and output. Because of the sluggish exchange of $Rb^{86}$ and of potassium between the plasma and erythrocytes, $Rb^{86}$ was not "washed" out of the red blood cells and, therefore, the regression curves showed no detectable change in their rates of decline. Thus, it is possible for the concentration of $Rb^{86}$ in the plasma to vary at least quantitatively independently of the red blood cells and other cells of the body. Sudden changes in the steady state of potassium metabolism, including intake and output, pose special problems for the tracer method. When the state of potassium metabolism was steady, with the rate of intake and output constant for a fairly long period of time, the time course of the concentration of $Rb^{86}$ in the plasma and erythrocytes tended to become parallel. This would be expected when the exchanges of $Rb^{86}$ between plasma and erythrocytes had sufficient time to follow each other. This situation most probably holds for potassium also. Thus, as indicated earlier in this report, it is necessary to be cognizant of the compartment sampled for measurements when interpreting data concerned with tracer studies.

Figs. 6, 7, 8, 9, 15, and 16 show the concentration of $Rb^{86}$ in the erythrocytes to reach about twenty or thirty times that in the plasma. This finding is in further support of the fact that $Rb^{86}$ enters the metabolic pool of potassium because the intracellular concentration of potassium in erythrocytes is essentially twenty times the concentration in the plasma. The higher proportion of $Rb^{86}$ than $K^{39}$ in the erythrocytes than in the plasma was due most probably to a lag in the transfer of $Rb^{86}$ from the erythrocytes back into the plasma as $Rb^{86}$ was lost from the plasma. This lag is a manifestation of relatively slow rates of exchange between the plasma and the erythrocytes. Subject 602 (Fig. 7) was the only subject who failed to reveal such a lag, probably because the rate of exchange of $Rb^{86}$ between the plasma and the

erythrocytes was relatively rapid. From comparison of the values in Figs. 15 and 16 for Subjects 601 and 602 with the curves shown in Figs. 6 and 7, it becomes evident that the $Rb^{86}$ in the erythrocytes and plasma follow each other more closely with time for control Subject 602 than for control Subject 601. Fig. 16 shows in greater detail the daily variations and differences between these two subjects, supporting better tracing of potassium with $Rb^{86}$ in Subject 602 than in Subject 601.

## SUMMARY

Radioactive rubidium, $Rb^{86}$, was employed in an attempt to trace potassium in the body of man with and without chronic congestive heart failure. The data certainly apply to the tracing of rubidium and study of rubidiokinetic processes. Although $Rb^{86}$ does not trace potassium precisely, it apparently traces potassium sufficiently well to permit generalizations concerning certain aspects of kaliokinetic phenomena for man. Statements rendered in this report concerning rates of turnover of potassium were based upon the assumption that $Rb^{86}$ traced potassium.

$Rb^{86}$ and $K^{42}$ employed simultaneously in one control subject and in another with moderately severe chronic congestive heart failure revealed that the two tracers behaved similarly during the first 120 minutes following the intravenous administration of the tracers. The partitioning of $K^{42}$ and $Rb^{86}$ in the intra- and extravascular compartments also was similar.

$Rb^{86}$ entered the erythrocytes relatively slowly. The concentration increased for five to eight days following the intravenous administration before it began to decline. Equilibrium of distribution of $Rb^{86}$ certainly was not established in the body by this time and apparently not at any time during the entire period of study.

The biologic decay rates were defined directly for $Rb^{86}$ and may be applied, with reservations, indirectly for potassium. Approximately ten to thirty-seven days were required for the concentration of $Rb^{86}$ in the plasma to reach one-half the concentration obtained five days following the intravenous administration. From about 59 to 188 days were required for one-half of the administered $Rb^{86}$ to be excreted in the urine and 39 to 134 days for one-half to be excreted in the urine and feces combined.

Because of the lag in the loss of $Rb^{86}$ from the erythrocytes, the distribution of the tracer and $K^{39}$ between the erythrocytes and plasma was disproportionate. The magnitude of this difference in distribution between plasma and erythrocytes varied from day to day and among the four subjects studied.

The turnover rates were not altered in a predictable fashion by variations in dietary intake of potassium. The rate of fall of concentration of $Rb^{86}$ in the plasma was influenced more than that for the erythrocytes or body cells as a whole as measured by the rate of excretion of the tracer.

Study of the concentration of $Rb^{86}$ in the plasma does not necessarily indicate the behavior of the tracer or nontracer in the cells, especially when the plasma is studied for only a short period of time. Therefore, applications of indirect observations, such as the measurement of $Rb^{86}$ and potassium in

the plasma, to certain aspects of intracellular rates of turnover and metabolism of potassium, must be made cautiously. The more compartments studied, the more clearly are rubidiokinetic and kaliokinetic phenomena defined.

The applications of these studies to atomic warfare and other phases of physiology concerning radioactive potassium or rubidium, such as radiation safety, were indicated.

## REFERENCES

1. Love, William D., and Burch, George E.: A Comparison of Potassium$^{42}$, Rubidium$^{86}$, and Cesium$^{134}$ as Tracers of Potassium in the Study of Cation Metabolism of Human Erythrocytes in Vitro, J. LAB. & CLIN. MED. **41**: 351, 1953.
2. Hodgkin, A. L.: The Effect of Potassium on the Surface Membrane of an Isolated Axon, J. Physiol. **106**: 319, 1947.
3. Cowan, S. L.: The Action of Potassium and Other Ions on the Injury Potential and Action Current in Maia Nerve, Proc. Roy. Soc. London, s.B **115**: 216, 1934.
4. Wilbrandt, W.: The Effect of Organic Ions on the Membrane Potential of Nerves, J. Gen. Physiol. **20**: 519, 1937.
5. Sandow, A., and Mandel, H.: Effects of Potassium and Rubidium on the Resting Potential of Muscle, J. Cell & Comp. Physiol. **38**: 271, 1951.
6. Clark, A. J.: The Mode of Action of Potassium Upon Isolated Organs, J. Pharmacol. & Exper. Therap. **18**: 423, 1921.
7. Mann, P. J. G., Tennenbaum, M., and Quastel, J. H.: Acetylcholine Metabolism in the Central Nervous System, the Effects of Potassium and Other Cations on Acetylcholine Liberation, Biochem. J. **33**: 822, 1939.
8. Mitchell, P. H., Wilson, J. W., and Stanton, R. E.: The Selective Absorption of Potassium by Animal Cells, II. The Cause of Potassium Selection as Indicated by the Absorption of Rubidium and Cesium, J. Gen. Physiol. **4**: 141, 1921.
9. Follis, R. H.: Histological Effects in Rats Resulting From Adding Rubidium or Cesium to a Diet Deficient in Potassium, Am. J. Physiol. **138**: 246, 1943.
10. Roberts, R. B., Roberts, I. Z., and Cowie, D. B.: Potassium Metabolism in Escherichia Coli. II. Metabolism in the Presence of Carbohydrates and Their Metabolic Derivatives, J. Cell & Comp. Physiol. **34**: 259, 1949.
11. Lorente de Nó, Rafael: A Study of Nerve Physiology, Studies From the Rockefeller Institute for Medical Research **131**: 31, 1947.
12. Love, W. D., and Burch, G. E.: Plasma and Erythrocyte Sodium and Potassium Concentrations in a Group of Southern White and Negro Blood Donors, J. LAB. & CLIN. MED. **41**: 258, 1953.
13. Schales, O., and Schales, S.: A Simple and Accurate Method for the Determination of Chloride in Biologic Fluids, J. Biol. Chem. **140**: 879, 1941.
14. Burch, George, Reaser, Paul, Ray, Thorpe, and Threefoot, Sam: A Method of Preparing Biologic Fluids for Counting of Radioelements, J. LAB. & CLIN. MED. **35**: 626, 1950.
15. Burch, George, Reaser, Paul, and Cronvich, James: Rates of Sodium Turnover in Normal Subjects and in Patients With Congestive Heart Failure, J. LAB. & CLIN. MED. **32**: 1169, 1947.
16. Unpublished observations with $Na^{22}$.
17. Ray, C. T., Burch, G. E., and Threefoot, S. A.: Biologic Decay Rates of Chloride in Normal and Diseased Man Determined With Long-Life Radiochloride, $Cl^{36}$, J. LAB. & CLIN. MED. **39**: 673, 1952.
18. Threefoot, S. A., Ray, C. T., Burch, G. E., Cronvich, J. A., Milnor, J. P., Overman, W., and Gordon, W.: Concentration-Time Course in the Plasma of Man of Radiomercury Introduced as a Mercurial Diuretic, J. Clin. Invest. **28**: 661, 1949.
19. Gellhorn, A., Merrell, M., and Rankin, R. M.: Rate of Transcapillary Exchange of Sodium in Normal and Shocked Dogs, Am. J. Physiol. **142**: 407, 1944.
20. Merrell, M., Gellhorn, A., and Flexner, L. B.: Studies on Rates of Exchange of Substances Between Blood and Extravascular Fluid; Exchange of Sodium in the Guinea Pig, J. Biol. Chem. **153**: 83, 194.
21. Boyle, P. J., and Conway, E. J.: Potassium Accumulation in Muscle and Associated Changes, J. Physiol. **100**: 1, 1941.
22. Burch, G. E., Ray, C. T., and Threefoot, S. A.: Estimation of the Time of Equilibrium of Distribution of Long-Life Radiochloride and Radiosodium in Man With and Without Chronic Congestive Heart Failure, Acta med. scandinav. **142**: 329, 1952.
23. Love, W. D., Romney, R. B., and Burch, G. E.: A Comparison of the Distribution of Potassium and Exchangeable Rubidium in the Organs of the Dog, Using $Rb^{86}$, Circulation Res. **2**: 112, 1954.

24. Burch, G. E., Threefoot, S. A., and Ray, C. T.: Rates of Turnover and Biologic Decay of Chloride and Chloride Space in the Dog Determined With the Long-Life Isotope, $Cl^{36}$, J. LAB. & CLIN. MED. **35**: 331, 1950.
25. Threefoot, Sam, Burch, George, and Reaser, Paul: The Biologic Decay Periods of Sodium in Normal Man, in Patients With Congestive Heart Failure, and in Patients With the Nephrotic Syndrome as Determined by $Na^{22}$ as the Tracer, J. LAB. & CLIN. MED. **34**: 1, 1949.
26. Threefoot, S. A., Burch, G. E., and Ray, C. T.: Chloride "Space" and Total Exchanging Chloride in Man Measured With Long-Life Radiochloride, $Cl^{36}$, J. LAB. & CLIN. MED. **42**: 16, 1953.
27. Burch, G. E., Ray, C. T., and Threefoot, S. A.: Some Theoretic Considerations of Electrolyte Space Measured by the Tracer Method in Intact Man, J. LAB. & CLIN. MED. **42**: 34, 1953.
28. Threefoot, S. A., Ray, C. T., and Burch, G. E.: Study of the Use of $Rb^{86}$ as a Tracer for the Measurement of $Rb^{86}$ and $K^{39}$ Space and Mass in Intact Man With and Without Congestive Heart Failure, J. LAB. & CLIN. MED. **45**: 395, 1955.
29. Ray, C. T., Threefoot, S. A., and Burch, G. E.: The Excretion of Radiorubidium, $Rb^{86}$, Radiopotassium, $K^{42}$, Sodium and Chloride by Man With and Without Congestive Heart Failure, J. LAB. & CLIN. MED. **45**: 408, 1955.
30. Corsa, Leslie, Jr., Olney, John M., Jr., Steenburg, Richard W., Ball, Margaret R., and Moore, Francis D.: The Measurement of Exchangeable Potassium in Man by Isotope Dilution, J. Clin. Invest. **29**: 1280, 1950.
31. Aikawa, Jerry K., Felts, John H., Jr., and Harrell, George T.: Isotopic Studies of Potassium Metabolism in Diabetes, J. Clin. Invest. **32**: 15, 1953.

# STUDY OF THE USE OF $Rb^{86}$ AS A TRACER FOR THE MEASUREMENT OF $Rb^{86}$ AND $K^{39}$ SPACE AND MASS IN INTACT MAN WITH AND WITHOUT CONGESTIVE HEART FAILURE

S. A. Threefoot, M.D., C. T. Ray, M.D., and G. E. Burch, M.D.*
New Orleans, La.

IT HAS been shown that rubidium enters the metabolic pool of potassium,[1-11] and since the half-life of $K^{42}$ is too short for prolonged observations, $Rb^{86}$ was employed to trace potassium over long periods of time. It was shown in previous studies that $Rb^{86}$ traces potassium sufficiently well to provide data of importance concerning metabolic processes involving potassium.[1, 2, 12, 13] During the course of prolonged tracer studies with $Rb^{86}$ to investigate aspects of potassium metabolism in patients with congestive heart failure and in control subjects, experiments were designed to provide information concerning space and mass in these subjects. Unfortunately, as indicated in a previous report,[12] equilibrium of distribution of $Rb^{86}$ was never established in these subjects. This is probably due, in large part, to the facts that rubidium and potassium compartments are principally intracellular and large and that there is a lag in the redistribution of these elements between the intracellular and extracellular compartments when the steady state of potassium metabolism is altered. Although similar difficulties arose with the use of $Na^{22}$ and $Cl^{36}$ in the measurements of sodium and chloride spaces and masses, they were not as great since these elements are primarily extracellular and the extracellular compartment was sampled. However, the problems of lag, sequestration, and failure to enter compartments apply to these elements as well as to rubidium and potassium.[14, 15]

In spite of these limitations, the data were analyzed to learn the time course of variations in space of $Rb^{86}$, indirectly of space and mass of $K^{39}$, and aspects of the problems and difficulties concerned with the use of rubidium and potassium tracers.

It should be indicated that, on the basis of data from this laboratory and elsewhere, it is assumed that $Rb^{86}$ traces potassium sufficiently well to provide significant indirect information regarding variations in magnitude of potassium space and mass as well as rubidium space. It is not contended that rubidium and potassium spaces are identical or that mass of potassium calculated from rubidium data represents the precise value of the amount of potassium in the body. Nevertheless, in spite of the limitations, the data provide information of importance related to the state of potassium metabolism in control

Aided by grants from the Public Health Service, H-143, United States Atomic Energy Commission, AT-(40-1)-233, and the Mrs. E. J. Caire Fund for Research in Heart Disease.
Received for publication Oct. 20, 1954.

*From the Department of Medicine, Tulane University School of Medicine, and Charity Hospital of Louisiana at New Orleans, La.

subjects and in patients with congestive heart failure unavailable by other methods of study. When space and mass of potassium and space of $Rb^{86}$ were estimated by sampling an intracellular compartment, erythrocytes, the data were more reliable than when estimations were obtained from measurements of the extracellular compartment, plasma. Furthermore, these data provide additional information concerning the magnitude of the errors that may be obtained based on the assumption that equilibrium of distribution has been achieved.

## METHODS AND MATERIALS

Radiorubidium ($T_{½}$ 19.5 days), as $Rb^{86}Cl$, was administered intravenously to 3 subjects—one control subject, one with moderate congestive heart failure, and one with severe congestive heart failure (Table I). The dose of $Rb^{86}$ was $2 \times 10^8$ CPM ($\approx$ 1.0 mc.). After study of the rapid disappearance of $Rb^{86}$ from the plasma,[12] the subjects were returned to a special ward in Charity Hospital where they were kept under constant surveillance by special nurses who controlled dietary intake* and medications, measured body weight daily, and collected all biologic fluids and excreta. A sample of heparinized blood was collected each morning at approximately 9 A.M. Each specimen of urine and feces was collected in a separate container and the quantity and time each was passed was recorded. Each sample was assayed separately and then all collections for each 24-hour period were pooled and reassayed as a daily sample. Special fluids such as vomitus, sputum, and ascitic and pleural fluids were collected and studied. The diets were not changed except that known quantities of potassium choride and other substances were added as indicated in the illustrations to follow. Determinations of the sodium, potassium, and chloride were made in all body fluids which were studied for $Rb^{86}$. Sodium and potassium were determined by the Beckman flame photometer, and chloride by the method of Schales.[16] The radioactivity was determined by the method previously described.[17]

TABLE I. CLINICAL DATA ON THREE MALE SUBJECTS STUDIED

| SUBJECT | AGE (YR.) | INITIAL BODY WEIGHT (KG.) | DIAGNOSIS | DAYS OF OBSERVATION | SEVERITY OF EDEMA |
|---|---|---|---|---|---|
| 602 | 57 | 70.5 | (Control subject) Anxiety state | 42 | 0 |
| 603 | 39 | 55.8 | CHF moderate to severe, rheumatic heart disease, neurofibromatosis | 44 | 2+ |
| 604 | 45 | 55.5 | Cor pulmonale, severe CHF | 38 | 3+ |

An aliquot of the heparinized blood was centrifuged at 3,000 r.p.m. for 30 minutes as quickly as possible after collection. The hematocrit was determined and the plasma separated for counting and chemical determinations. Another aliquot of whole blood was laked by freezing and thawing for counting and the chemical determinations. From these data, the $Rb^{86}$ and potassium contents of the erythrocytes were calculated using the following equation:

$$C_R = \frac{C_{WB} - (1 - H)\ C_P}{H} \qquad (1)$$

where
$C_R$ = the concentration of $Rb^{86}$ or K in erythrocytes,
$C_{WB}$ = concentration of $Rb^{86}$ or K in the whole blood,
$C_P$ = concentration of $Rb^{86}$ or K in the plasma and
H = the hematocrit.

*For these studies, the cooperation of Sister Euphemia and Mrs. Betty Doolittle of the dietary department of Charity Hospital is greatly appreciated.

No corrections were made for trapped plasma which amounted to 2 to 3 per cent of the hematocrit value.

Since the amount of $Rb^{86}$ remaining in the blood on any day and the concentration of $Rb^{86}$ in the plasma and erythrocytes were measured, the rubidium space in *milliliters of plasma compartment volume equivalents* and in *milliliters of erythrocyte compartment volume equivalents* were calculated by the isotope dilution method (Equation 2).

The symbols and equations employed in these studies are as follows:

$S_o$ = calculated tracer ($Rb^{86}$) space, or volume, expressed in *sampled compartment volume equivalents* at $t_o$, i.e., that volume which the tracer ($Rb^{86}$) would occupy had it existed in all compartments at a concentration equal to that in the sampled compartment. In this paper the sampled compartment was either plasma, indicated by the symbol without any subscript, or erythrocytes, indicated by the subscript "R," e.g., $S_{R_o}$.

$S_t$ = calculated tracer ($Rb^{86}$) space, in sampled compartment volume equivalents at any time t.

$t_o$ = time of administration of the tracer.

$M_o$ = total *calculated* exchanging nontracer ($K^{39}$) mass, in grams, in the body at $t_o$, calculated from the plasma values. $M_{R_o}$ is that calculated from the R.B.C. values.

$M_t$ = total actual exchanging nontracer ($K^{39}$) mass in the body, in grams, at any time t.

$M_T$ = calculated total nontracer ($K^{39}$) mass in the body, in grams, i.e., any total mass obtained by means of calculation at any time t. $M_{R_T}$ indicates that calculated from R.B.C. values.

$CM_o$ = concentration of nontracer ($K^{39}$) in sampled compartment, in grams per milliliter, at $t_o$. $CM_{R_o}$ is that in the R.B.C.

$CM_t$ = concentration of nontracer ($K^{39}$) in the sampled compartment, in grams per milliliter, at any time t. $CM_{R_t}$ is that in the erythrocytes in grams per milliliter.

$N_o$ = total amount of tracer ($Rb^{86}$) administered, in counts per minute (CPM), at $t_o$.

$N_K$ = output of tracer ($Rb^{86}$) in CPM per day.

$N_T$ = total amount of tracer ($Rb^{86}$) remaining in the body in CPM, at any time t.

$CN_o$ = extrapolated concentration of tracer ($Rb^{86}$) in sampled compartment, in CPM per milliliter, at $t_o$. $CN_{R_t}$ is that in the R.B.C.

I = intake of nontracer ($K^{39}$) in grams per day.

K = output of nontracer ($K^{39}$) in grams per day.

BW = body weight in grams.

From the foregoing definitions, these equations must follow:

$$S_o = \frac{N_o}{CN_o} \quad ; \quad S_{R_o} = \frac{N_o}{CN_{R_o}} \quad . \quad (2)$$

$$S_t = \frac{N_t}{CN_t} \quad ; \quad S_{R_t} = \frac{N_t}{CN_{R_t}} \quad . \quad (3)$$

$$M_o = S_o \times CM_o \quad ; \quad M_{R_o} = S_{R_O} \times CM_{R_O} \quad . \quad (4)$$

$$M_T = S_t \times CM_t \quad ; \quad M_{R_T} = S_{R_t} \times CM_{R_t} \quad . \quad (5)$$

Another equation for the calculation of $M_T$ without the direct calculation of space may be expressed as:

$$M_T = \frac{\text{Amount of tracer remaining in the body at time t}}{\text{Specific activity at time t}}$$

$$= \frac{N_T}{CN_t/CM_t} \quad . \quad (6)$$

TABLE II. AVERAGE "SPACE" AND "MASS" CALCULATED FROM R.B.C. AND FROM PLASMA ON LOW AND HIGH POTASSIUM DIETS

| | | | CALCULATED FROM R.B.C. | | | | | CALCULATED FROM PLASMA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SUBJECT | DAYS OF AVG. INTAKE | AVERAGE BODY WEIGHT (GM.) | AVERAGE CALCULATED SPACE $S_R$ (ML. R.B.C. EQ.) | AVERAGE SPACE IN R.B.C. EQ. (% BW) | AVERAGE R.B.C. K CONC. $CM_R$ (GM./ML.) | AVERAGE CALCULATED K MASS $S_R \times CM_R$ (GM.) | AVERAGE CALCULATED K MASS R.B.C. EQ. AS % 6BW* | AVERAGE CALCULATED SPACE $S_t$ (ML. PLASMA EQ.) | AVERAGE SPACE IN PLASMA EQ. % BW | AVERAGE PLASMA K CONC. $CM_t$ (GM./ML.) | AVERAGE CALCULATED K MASS $S_t \times CM_t$ (GM.) | AVERAGE CALCULATED K MASS PLASMA EQ. AS % BW* |
| *A. Low potassium intake—average 68 meq. per day* | | | | | | | | | | | | |
| 602 | 21 | 69,684 | 38,076 | 54.6 | 0.00359 | 136.7 | 0.196 | 1,026,111 | 1,473 | 0.000167 | 171.3 | 0.245 |
| 603 | 22 | 55,945 | 27,551 | 49.1 | 0.00379 | 104.3 | 0.186 | 868,128 | 1,552 | 0.000157 | 136.3 | 0.243 |
| 604 | 23 | 55,476 | 24,178 | 43.5 | 0.00376 | 91.0 | 0.164 | 662,688 | 1,195 | 0.000175 | 115.5 | 0.207 |
| *B. High potassium intake—average 124 meq. per day†* | | | | | | | | | | | | |
| 602 | 6 | 69,291 | 43,811 | 63.2 | 0.00347 | 152.1 | 0.219 | 1,164,129 | 1,680 | 0.000163 | 189.7 | 0.274 |
| 603 | 7 | 57,301 | 32,045 | 55.9 | 0.00373 | 119.5 | 0.208 | 1,236,172 | 2,157 | 0.000147 | 181.7 | 0.317 |
| 604 | 3 | 57,431 | 26,116 | 45.4 | 0.00377 | 98.5 | 0.171 | 650,109 | 1,132 | 0.000236 | 153.4 | 0.267 |

*This column × 10 = Gm./kg. body weight.

†The values calculated from data obtained late in study and counts lower. This and small number diminishes the accuracy of these values especially for the plasma equivalent calculations.

TABLE III. CALCULATION OF $Rb^{86}$ "SPACE" AND POTASSIUM "MASS" AT $t_o$ IN THREE HUMAN SUBJECTS

A. IN R.B.C. EQUIVALENTS

| SUBJECT | AMOUNT INJECTED $N_o$ (CPM) | R.B.C. CONC. $Rb^{86}$ $CN_{R_o}$ (CPM/ML.) | $Rb^{86}$ SPACE $S_{R_o} = N_o/CN_{R_o}$ (ML. R.B.C. EQ.) | BODY WEIGHT (GM.) | $Rb^{86}$ SPACE (% BW) | $K^{39}$ CONC. R.B.C. $CM_{R_o}$ (GM./ML.) | CALCULATED TOTAL K MASS $M_{R_o} = S_{R_o} \times CM_{R_o}$ (GM.) | $K^{39}$ MASS AS % BW |
|---|---|---|---|---|---|---|---|---|
| 602 | 200,000,000 | 5,100 | 39,216 | 70,484 | 56.34 | 0.00369 | 144.71 | 0.2053 |
| 603 | 200,000,000 | 7,300 | 27,397 | 56,100 | 48.84 | 0.00334 | 91.62 | 0.1633 |
| 604 | 200,000,000 | 6,000 | 33,333 | 55,700 | 59.84 | 0.00374 | 124.7 | 0.2239 |

B. IN SERUM EQUIVALENTS

| SUBJECT | AMOUNT INJECTED $N_o$ (CPM) | SERUM CONC. $Rb^{86}$ CN (CPM/ML.) | $Rb^{86}$ SPACE $S_o = N_o/CN_o$ (ML. SERUM EQ.) | BODY WEIGHT (GM.) | $Rb^{86}$ SPACE (% BW) | $K^{39}$ CONC. SERUM $CM_o$ (GM./ML.) | CALCULATED TOTAL K MASS $M_o = S_o \times CM_o$ | $K^{39}$ MASS AS % BW |
|---|---|---|---|---|---|---|---|---|
| 602 | 200,000,000 | 310 | 645,161 | 70,484 | 915.3 | 0.0001607 | 103.68 | 0.1471 |
| 603 | 200,000,000 | 290 | 689,655 | 56,100 | 1,229.3 | 0.000156 | 107.59 | 0.1918 |
| 604 | 200,000,000 | 290 | 689,655 | 55,700 | 1,238.2 | 0.0001794 | 123.72 | 0.2386 |

The applications and the limitations of these methods and these equations have been discussed elsewhere.[14, 15] In order to estimate the potassium content of the body at any time t, it was assumed that the $Rb^{86}$ traced the potassium. For purposes of comparison, calculated space of potassium was expressed in plasma or erythrocyte volume equivalents as a percentage of body weight. Since there are much larger errors involved in the assumption that equilibrium of distribution occurs early, all average values of space and mass were calculated from data obtained at least five days following administration of the tracer.

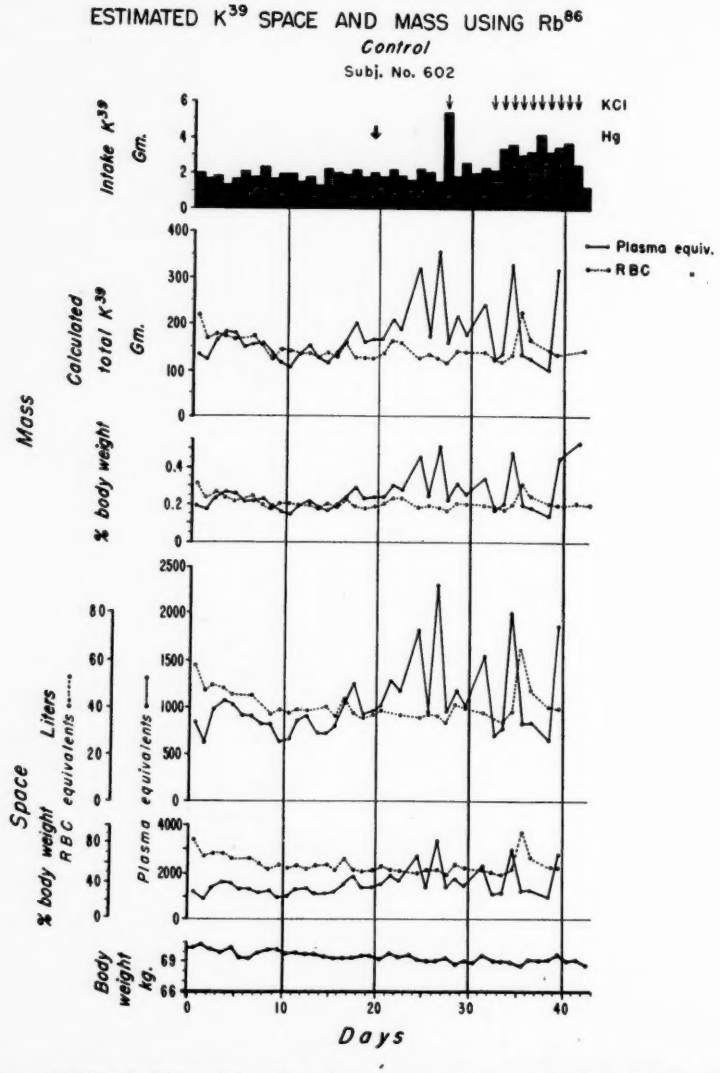



Fig. 1.—Control Subject 602. The time course of the potassium intake and drugs; the calculated exchanging potassium in grams and percentage of body weight; calculated space in liters and percentage body weight; and the body weight in kilograms. The time course curves of calculated total mass, mass as percentage of body weight, space and space as percentage of body weight have been calculated using the concentration of $Rb^{86}$ in the erythrocytes and in the plasma. After the first week had elapsed these parameters calculated from data on erythrocytes were more stable than those derived from plasma values. In addition to the marked changes produced by increasing the intake of potassium there was a gradual increase in these calculated values obtained from plasma determinations.

## RESULTS

The results are summarized in Tables II and III and Figs. 1, 2, and 3.

Approximately nine days were required for the calculated rubidium space expressed in erythrocyte (R.B.C.) volume equivalents and for the calculated mass expressed in R.B.C. mass equivalents to approach relatively constant values for control Subject 602 (Fig. 1). While for Subject 603 (Fig. 2) with moderate congestive heart failure, approximately five days were required for this

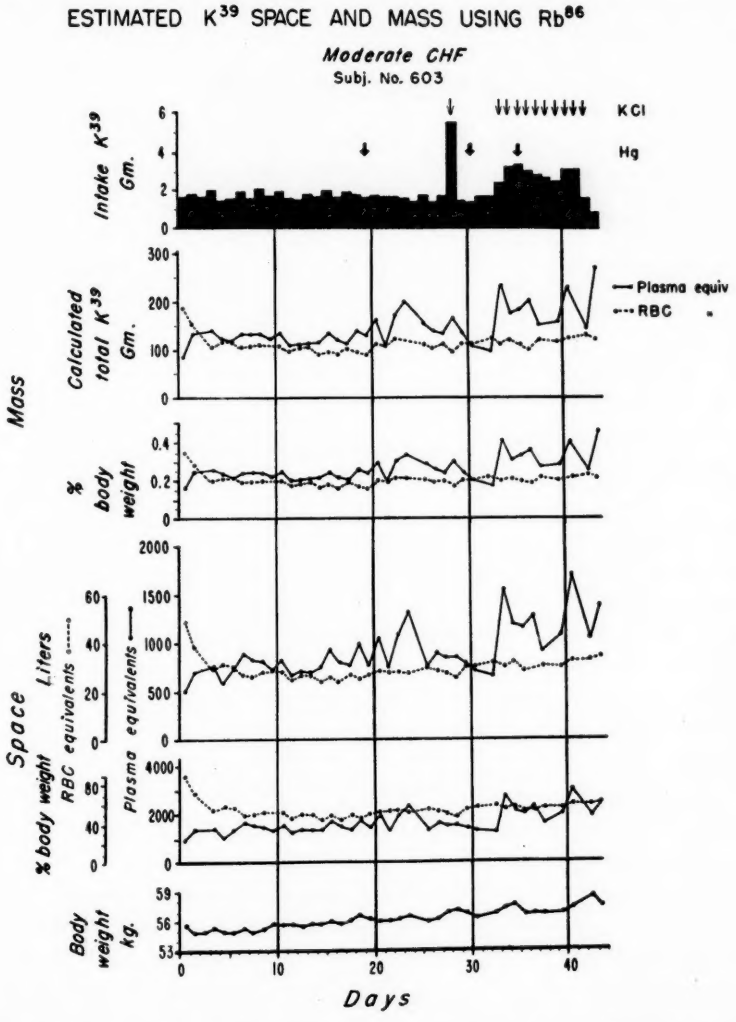



Fig. 2.—Subject 603 with moderately severe congestive heart failure. Consult legend for Fig. 1 for details.

to occur and about three days for Subject 604 (Fig. 3) with severe congestive heart failure. Thus, apparently this length of time was required for the red cells to accumulate the major portion of their share of the isotope. The same would be true for potassium and indicates the inability to determine ac-

curately, with biologically safe doses, the potassium space and mass with the short half-life isotope $K^{42}$. Although the most seriously ill patient approached a steady state of the calculated values most rapidly, this does not necessarily indicate that there is a more rapid exchange of potassium between the plasma and cellular compartments in all such patients.

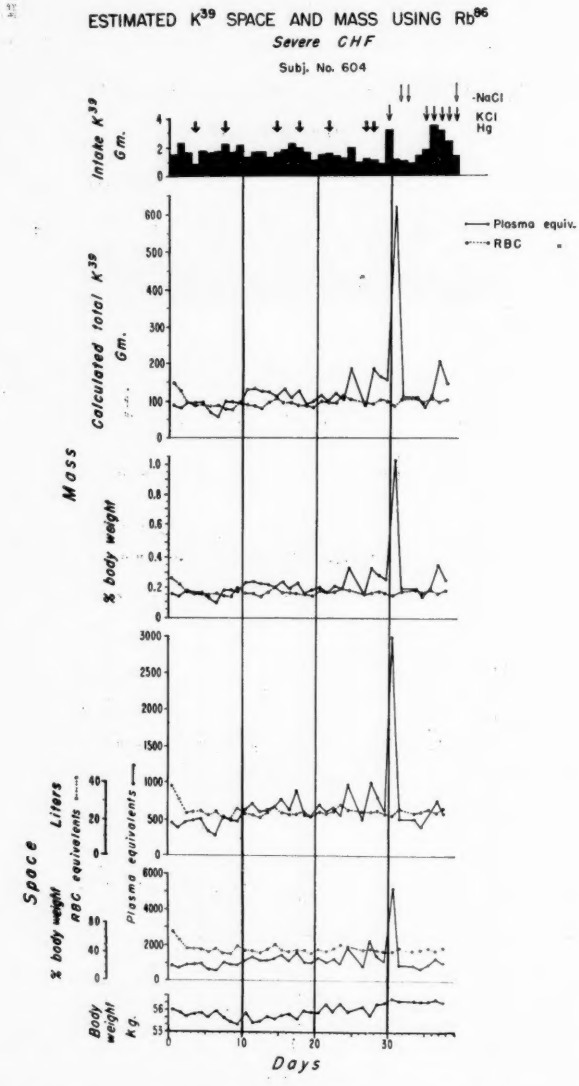



Fig. 3.—Subject 604 with severe congestive heart failure. Consult legend for Fig. 1 for details.

Calculated space and mass values expressed in erythrocyte volume and mass equivalents exhibited much less variation than those expressed in plasma volume and mass equivalents (Figs. 1, 2, and 3). This demonstrates the errors introduced by sampling a compartment which contains a relatively small portion of the total mass of nontracer and tracer, such as the plasma, when at-

tempting to estimate the total space and mass of elements which are primarily intracellular, as are potassium and rubidium. Thus, when the greater portion of the nontracer is intracellular, an intracellular compartment such as erythrocytes should be sampled in order to offer more reliable information about space and mass and to record turnover data of the body as a whole. It may be argued that skeletal muscle would be a more representative tissue, but it does not lend itself for frequent (hourly or daily) biopsy whereas the erythrocytes do. When tracing substances which are predominantly extracellular, such as sodium and chloride, the extracellular compartment should be the primary one sampled.[14, 15, 18, 19]

In general, except for the first few days, calculated space and mass, expressed in erythrocyte volume and mass equivalents, were smaller than the values of calculated space and mass expressed in plasma volume and mass equivalents. This was the result of a progressive rise in the values of space and mass expressed in plasma equivalents with essentially no change in those values expressed in erythrocyte equivalents, due to a lag in change in concentration of the tracer in the erythrocytes as compared to that in the plasma. There was a more rapid decline in plasma concentration of the tracer than could be accounted for by excretion from the body. In addition, because of the lag in the decline in concentration in the erythrocytes, the plasma contributed a disproportionately greater amount of the isotope excreted. Incidentally, it is well to point out that there were no marked changes in body weight during these experiments.

The mean values for calculated space and mass are summarized in Table II. It is evident from this table that the calculations for space and mass with and without correction for body weight indicate a greater mass and space for the normal subject than for the two subjects with congestive heart failure, the patient with severe congestive heart failure having the smallest values. Because of the more dependable values obtained in terms of erythrocyte space and mass equivalents and because of the fact that the extracellular mass of potassium is known to be small, the differences between the control and the patients with congestive heart failure are most probably reliable. However, the absolute values are probably not exact. It is interesting to note that the concentration of $Rb^{86}$ in the erythrocytes was twenty to thirty times that in the plasma and, as would be expected, the space in erythrocyte volume equivalents was $\frac{1}{20}$ to $\frac{1}{30}$ that of plasma volume equivalents.

Table III summarizes calculations of space and mass at the time of injection ($t_o$). This was done by extrapolating to $t_o$ from the concentration-time course curves after the first seven days had elapsed. Such an analysis is not likely to be dependable within the first week when the concentration in the intracellular compartment is still rising and that of the extracellular compartment is declining. Extrapolation from the concentration-time course curve of the major compartment (intracellular such as R.B.C.) would be expected to be more dependable than extrapolation from that of the extracellular compartment (plasma). Realizing the inaccuracies of space and mass measurements

by the $Rb^{86}$ technique when equilibrium of distribution was not fully established, it is interesting to note for the three subjects, that when expressed in erythrocyte equivalents, the average space and mass values for the entire period of study were remarkably similar to those at $t_o$ (Tables II and III).

No consistent change in calculated space and mass was produced by administration of mercurial diuretics to any of the subjects (Figs. 1, 2, and 3). This is quite different from responses observed for chloride space and mass. Calculated space and mass expressed in erythrocyte space and mass equivalents were increased slightly by dietary supplements of potassium as potassium chloride (Figs. 1, 2, and 3). The calculated values expressed in plasma volume and mass equivalents were not reliable because the counts were too near background at the time the diet was varied.

## GENERAL DISCUSSION

With the use of radiorubidium, because of its long half-life, it was possible to observe continuously for a period of fifty days the time course of rubidium space and potassium space and mass for the body as a whole. Such observations were not possible with $K^{42}$. These data indicate that space and mass fluctuate considerably and that a single measurement is not dependable as a representation of the magnitude of space and mass. $Rb^{86}$, unfortunately, is chemically and physically different from potassium but sufficiently similar in biologic behavior so that the experiments did provide data sufficiently reliable to indicate certain aspects and problems concerning potassium metabolism. $Rb^{86}$ was certainly traced in these experiments, but because the ordinary form of rubidium was not determined it is not known how accurately rubidium was traced.

Realizing the limitations of any implications concerning potassium metabolism from these studies with $Rb^{86}$, certain generalizations can most probably be made although errors in absolute values exist.

For example, it is evident from these studies and as indicated in a previous report that it is not possible to trace adequately and to measure the time-course variations in potassium space and mass by sampling an extracellular compartment such as plasma. This is due to the fact that the major compartment is intracellular and changes in the plasma compartment do not adequately reflect changes in the major compartment because of lags in exchange between the two compartments. Variations in dietary intake of the nontracer ($K^{39}$) may measurably influence the plasma compartment without causing measurable changes in the intracellular compartment. Furthermore, equilibrium of distribution was never established throughout the body as a whole, especially among the plasma or extracellular compartment and intracellular compartments. On the other hand, sampling of a portion of the major compartment, such as erythrocytes, reflected more accurately the turnover phenomena of $Rb^{86}$ and $K^{39}$ and indicated the time-course variations in space and mass of $K^{39}$. Thus, this is comparable to sampling the major compartment such as

plasma when studying sodium and chloride turnover phenomena. It is not necessary at this time to discuss again such important factors as sequestration and differences in rates of exchange between minor and major compartments in studies of this type.[15] Interpretations of data derived from sampling of only one of many compartments should be made cautiously.

It is interesting to note that the calculated mass of $K^{39}$ obtained from the erythrocyte measurements approach those presented by Shohl[20] and obtained from analyses of human bodies. It should be remembered, however, that although the measurements derived from studies of the erythrocytes are more accurate than those derived from study of the plasma, the degree of accuracy is still unknown. Studies of the distribution of $Rb^{86}$ and $K^{39}$ in various organic systems[2] have demonstrated that the $Rb^{86}$ to $K^{39}$ ratio in skeletal muscle is 1.08 times that in plasma or erythrocytes. Since skeletal muscle contains such a large portion of the mass of $Rb^{86}$ and potassium, any calculated space of $Rb^{86}$ would be 8 to 10 per cent larger than the potassium space and hence should result in a calculated exchanging potassium mass 8 to 10 per cent larger than the *"actual"* potassium mass of the body. Since organs such as liver yielded a $Rb^{86}$:$K^{39}$ ratio to that in the plasma which was even greater than 1.08, the error involved in the calculated mass obtained from plasma or erythrocyte ratios might be greater than 10 per cent.

These experiments showed that the two patients with congestive heart failure had a smaller calculated potassium space and mass (% BW) than the control subject. If the control subject is considered to represent the more normal values, then the subjects with congestive heart failure had a total potassium deficiency. It is not possible to be certain of this because the patients had edema which, because of its weight, could produce differences of the order observed. Muscle and other cellular mass was not measured and, therefore, it is possible that the intracellular concentration of potassium was normal and there might have been a simple reduction in cell mass. The two subjects with chronic congestive heart failure had been restricted in exercise whereas the control subject was a laborer. Clinical examination revealed a great difference in skeletal muscle mass between the control subject and the cardiac patients.

The time course curves of space and mass calculated from plasma values (Figs. 1, 2, and 3) indicated that increases in dietary intake of potassium tended to "wash" the isotope out of the plasma into the urine without complete permeation into the cellular compartments. An average man ingests and excretes each day an amount of potassium equivalent to the total content in the extracellular fluid compartment. Furthermore, these experiments suggest that a large portion of this intake flows through the extracellular fluids rapidly, resulting in a more rapid turnover in this compartment than in the intracellular compartment. The turnover in the extracellular compartment can be visualized as a very dynamic one compared with the more sluggish turnover in the intracellular compartment.

SUMMARY

An attempt was made to estimate $Rb^{86}$ space and potassium space and mass with the use of $Rb^{86}$. Apparently $Rb^{86}$, which permits prolonged studies, traces potassium sufficiently well to provide important information concerning potassium metabolism not obtainable through the use of short-life $K^{42}$.

Study of three subjects, one control and two with congestive heart failure, indicated that sampling of plasma or an extracellular fluid compartment would not necessarily provide sufficiently accurate measurements of $Rb^{86}$ space or potassium space and mass to make it possible to study these parameters by measuring the tracer and nontracer in the plasma. However, sampling of a portion (erythrocytes) of the major (intracellular) compartment of rubidium and potassium could provide values of $Rb^{86}$ space and potassium space and mass which are of sufficient reliability to provide valuable information concerning potassium and rubidium metabolism. When expressed as erythrocyte volume equivalents, the $Rb^{86}$ space was found to average 38,076 ml. for the control subject, 27,551 ml. for the subject with moderate congestive heart failure, and 24,178 ml. for the subject with severe congestive heart failure. These values were 54.6, 49.1, and 43.5 per cent of the body weight, respectively. The calculated total exchanging potassium mass obtained from measurements of the erythrocyte compartment was found to be 136.7 Gm. for the control subject, 104.3 Gm. for the subject with moderate congestive heart failure, and 91.0 Gm. for the subject with severe congestive heart failure. These values were 0.196, 0.186, and 0.164 per cent of the body weight. All of these values increased when the subjects were receiving supplemental dietary potassium chloride.

Although the concentration of potassium in the plasma and erythrocytes of the subject with severe congestive heart failure was not lower than the control subject, this subject had a smaller calculated space and mass. This difference, therefore, represented either less cell mass or deficiency of body potassium.

The time course curves of space and mass recorded for a period as long as fifty days revealed marked fluctuations and therefore indicate the unreliability of measurements of space and mass from a single observation.

REFERENCES

1. Love, William D., and Burch, George E.: A Comparison of Potassium$^{42}$, Rubidium$^{86}$, and Cesium$^{134}$ as Tracers of Potassium in the Study of Cation Metabolism of Human Erythrocytes in Vitro, J. Lab. & Clin. Med. **41:** 351, 1953.
2. Love, W. D., Romney, R. B., and Burch, G. E.: A Comparison of the Distribution of Potassium and Exchangeable Rubidium in the Organs of the Dog, Using $Rb^{86}$, Circulation Res. **2:** 112, 1954.
3. Hodgkin, A. L.: The Effect of Potassium on the Surface Membrane of an Isolated Axon, J. Physiol. **106:** 319, 1947.
4. Cowan, S. L.: The Action of Potassium and Other Ions on the Injury Potential and Action Current in Maia Nerve, Proc. Roy. Soc. London, s.B **115:** 216, 1934.
5. Wilbrandt, W.: The Effect of Organic Ions on the Membrane Potential of Nerves, J. Gen. Physiol. **20:** 519, 1937.
6. Sandow, A., and Mandel, H.: Effects of Potassium and Rubidium on the Resting Potential of Muscle, J. Cell. & Comp. Physiol. **38:** 271, 1951.

7. Clark, A. J.: The Mode of Action of Potassium Upon Isolated Organs, J. Pharmacol. & Exper. Therap. **18:** 423, 1921.
8. Mann, P. J. G., Tennenbaum, J., and Quastel, J. H.: Acetylcholine Metabolism in the Central Nervous System, the Effects of Potassium and Other Cations on Acetylcholine Liberation, Biochem. J. **33:** 822, 1939.
9. Mitchell, P. H., Wilson, J. W., and Stanton, R. E.: The Selective Absorption of Potassium by Animal Cells, II. The Cause of Potassium Selection as Indicated by the Absorption of Rubidium and Cesium, J. Gen. Physiol. **4:** 141, 1921.
10. Follis, R. H.: Histological Effects in Rats Resulting From Adding Rubidium or Cesium to a Diet Deficient in Potassium, Am. J. Physiol. **138:** 246, 1943.
11. Lorente de Nó, Rafael: A Study of Nerve Physiology. Studies From the Rockefeller Institute for Medical Research **131:** 31, 1947.
12. Burch, G. E., Threefoot, S. A., and Ray, C. T.: The Rate of Disappearance of $Rb^{86}$ from the Plasma, the Biologic Decay Rates of $Rb^{86}$, and the applicability of $Rb^{86}$ as a Tracer of Potassium in Man With and Without Chronic Congestive Heart Failure, J. LAB. & CLIN. MED. **45:** 371, 1955.
13. Ray, C. T., Burch, G. E., and Threefoot, S. A.: The Excretion of Radiorubidium, $Rb^{86}$, Radiopotassium, $K^{42}$, Sodium and Chloride by Man With and Without Congestive Heart Failure, J. LAB. & CLIN. MED. **45:** 408, 1955.
14. Threefoot, S. A., Burch, G. E., and Ray, C. T.: Chloride "Space" and Total Exchanging Chloride in Man Measured With Long-Life Radiochloride, $Cl^{36}$, J. LAB. & CLIN. MED. **42:** 16, 1953.
15. Burch, G. E., Ray, C. T., and Threefoot, S. A.: Some Theoretic Considerations of Electrolyte Space Measured by the Tracer Method in Intact Man, J. LAB. & CLIN. MED. **42:** 34, 1953.
16. Schales, O., and Schales, S.: A Simple and Accurate Method for the Determination of Chloride in Biologic Fluids, J. Biol. Chem. **140:** 879, 1941.
17. Burch, George, Reaser, Paul, Ray, Thorpe, and Threefoot, Sam: A Method of Preparing Biologic Fluids for Counting of Radioelements, J. LAB. & CLIN. MED. **35:** 626, 1950.
18. Burch, G. E., Threefoot, S. A., and Ray, C. T.: Rates of Turnover and Biologic Decay of Chloride and Chloride Space in the Dog Determined With the Long-Life Isotope, $Cl^{36}$, J. LAB. & CLIN. MED. **35:** 331, 1950.
19. Threefoot, Sam, Burch, George, and Reaser, Paul: The Biologic Decay Periods of Sodium in Normal Man, in Patients With Congestive Heart Failure, and in Patients With the Nephrotic Syndrome as Determined by $Na^{22}$ as the Tracer, J. LAB. & CLIN. MED. **34:** 1, 1949.
20. Shohl, Alfred T.: Mineral Metabolism, American Chemical Society Monograph No. 82, New York, 1939, Reinhold Publishing Corporation.

# THE EXCRETION OF RADIORUBIDIUM, $Rb^{86}$, RADIOPOTASSIUM, $K^{42}$, AND POTASSIUM, SODIUM, AND CHLORIDE BY MAN WITH AND WITHOUT CONGESTIVE HEART FAILURE

C. T. Ray, M.D., S. A. Threefoot, M.D., and G. E. Burch, M.D.*
New Orleans, La.

DURING experiments designed to determine the effectiveness with which radiorubidium, $Rb^{86}$, traces potassium,[1, 2] data were obtained which permitted the comparison of the excretion of radiorubidium and radiopotassium with that of potassium, sodium, and chloride. Similar comparisons of the excretion of radiochloride with that of chloride, potassium, and sodium have been made.[3] These experiments provided opportunity for additional comparisons of the excretion of the nontracer electrolytes as well as permitted further evaluation of radiorubidium as a tracer of potassium.

By use of the long-life tracer, $Rb^{86}$, it was possible to investigate some of the aspects of rubidiokinetic and kaliokinetic excretory phenomena which were not approachable without long-life tracers. These data indicate the influence of the normal physiologic state, congestive heart failure, variations in dietary intake of certain electrolytes, therapeutic procedures employed in the treatment of congestive heart failure and other factors on the excretion of radiorubidium, potassium, chloride, and sodium. Since the effects of these factors on the excretion of the various electrolytes were discussed in detail previously,[3] such discussions will not be repeated here except wherein they are pertinent to the comparison of the excretion of radiorubidium to that of the other electrolytes, especially potassium and radiopotassium, $K^{42}$. The various parameters to be presented include the excretion of radiorubidium as a percentage of that remaining in the body and the excretion of potassium as a percentage of that in the body as estimated from the space and mass of $Rb^{86}$ and potassium.[2] The experiments included a study of the routes of excretion other than the urine as well as dietary balance measurements of potassium, sodium, and chloride, urinary clearance rates, and "specific activity" in the materials sampled. Certain crucial observations were made utilizing simultaneous administrations of $K^{42}$ and $Rb^{86}$.

## METHODS AND MATERIALS

Six subjects were studied (Table I). One of the subjects (Subject 702) received intravenously $5 \times 10^7$ CPM ($\approx$ 0.25 mc.) of both $K^{42}$ and $Rb^{86}$ simultaneously and was observed only as long as the $K^{42}$ could be counted with accuracy. Samples of venous blood were collected at intervals of a few seconds immediately after injection and at

Aided by a Public Health Service Grant (H-143), the Mrs. E. J. Caire Fund for Research in Heart Disease, and a grant from the United States Atomic Energy Commission, AT-(40-1)-233.

Received for publication Oct. 20, 1954.

*From the Department of Medicine, Tulane University School of Medicine, and Charity Hospital of Louisiana at New Orleans, La.

progressively increasing intervals of time until two hours had elapsed. Samples of urine were collected by means of a bladder catheter as rapidly as the rate of urinary flow permitted and as nearly coincident with the samples of blood as possible. Another subject (Subject 703) received $5 \times 10^7$ CPM of both $K^{42}$ and $Rb^{86}$ orally and was followed for 45 days. Immediately after oral administration, samples of blood and urine were collected at 15-minute intervals for one hour and then hourly for five hours. The other four subjects, two control subjects and two with chronic congestive heart failure, received $2 \times 10^8$ CPM ($\approx$ 1.0 mc.) of $Rb^{86}$ Cl intravenously and were studied for 36 to 49 days under metabolically controlled conditions. Constant nursing surveillance was provided to control dietary intake* and medications and to assure collection of all excreta and biologic fluids. Each specimen of urine and feces was collected in a separate container and the quantity and time of passage of each were recorded. Each sample was assayed separately and then all collections for each 24-hour period were pooled and reassayed as a daily sample. Special fluids such a vomitus, sputum, and ascitic and pleural fluids were also collected and studied. A sample of blood was obtained at approximately 9 A.M. each day. The diets were not changed except for the addition of known quantities of potassium chloride and other substances as indicated in the illustrations to follow. Determinations of sodium, potassium, and chloride were made on all body fluids which were studied for $Rb^{86}$, except the stools, which were assayed only for $Rb^{86}$. Sodium and potassium were determined by the Beckman flame photometer and chloride by the method of Schales and Schales.[4] Radioactivity was measured by the method previously described.[5]

Calculations of the excretion as a percentage of the $Rb^{86}$, $K^{42}$, or $K^{39}$ remaining in the body to be excreted were carried out by the methods previously described for chloride.[2, 3] The "specific activity" reported for $Rb^{86}$ is not a true specific activity but is a ratio of the concentration of $Rb^{86}$ to that of $K^{39}$ in the various fluids and compartments studied. The calculations of clearance ( $\frac{UV}{B}$ = ml./min. cleared*) were obtained for each 24-hour period.

*U = concentration per ml. in the urine,
V = urinary flow in ml./min., and
B = concentration per ml. in plasma.

TABLE I. CLINICAL DATA

| SUBJECT | SEX | AGE (YR.) | INITIAL WEIGHT (KG.) | INJECTED DOSAGE (CPM) | DURATION OF STUDY | DIAGNOSIS |
|---|---|---|---|---|---|---|
| 601 | F | 34 | 78.2 | $2 \times 10^8$ $Rb^{86}$ | 38 days | Tuberculous adenitis, under treatment |
| 602 | M | 57 | 71.2 | $2 \times 10^8$ $Rb^{86}$ | 44 days | Anxiety state |
| 603 | M | 39 | 56.1 | $2 \times 10^8$ $Rb^{86}$ | 48 days | Congestive heart failure moderate to severe, rheumatic heart disease, neurofibromatosis |
| 604 | M | 45 | 55.7 | $2 \times 10^8$ $Rb^{86}$ | 40 days | Cor pulmonale, severe congestive heart failure, expired |
| 702 | F | 34 | 80.0 | $5 \times 10^7$ $Rb^{86}$<br>$5 \times 10^7$ $K^{42}$ | 2 hours | Hypertension, moderate congestive heart failure |
| 703 | F | 65 | — | $5 \times 10^7$ $Rb^{86}$<br>$5 \times 10^7$ $K^{42}$ | 45 days | Gastric neurosis |

## RESULTS

For convenience of presentation the data are divided into four parts: (1) simultaneous excretion of $Rb^{86}$ and $K^{42}$ during the first two hours following administration of the tracers, (2) recovery of the isotope by various

*Dietary supervision was provided by Sister Euphemia and Mrs. Betty Doolittle of the Dietary Department of Charity Hospital.

routes of excretion, (3) daily excretion and clearance of $Rb^{86}$ and excretion, clearance, and balance of potassium, chloride, and sodium, and (4) influence of various drugs on the excretion of $Rb^{86}$ and potassium.

1. *Simultaneous Excretion of $Rb^{86}$ and $K^{42}$ During the First Two Hours Following the Administration of the Tracers.—*

A. *Appearance time of the tracers in the urine:* The time of appearance of both tracers in the urine was between 50 seconds and 3 minutes 55 seconds for the subject who received simultaneously $K^{42}$ and $Rb^{86}$ intravenously and from whom rapid collections of urine were made by means of a bladder catheter. Another subject not included in this report had an appearance time of between 3 minutes 15 seconds and 4 minutes 55 seconds. These times of appearance were not significantly different from those described for $Na^{24}$ (Reference 6), $Hg^{203}$ (Reference 7), and $Cl^{36}$ (Reference 3). For the subject who received the tracers orally, urine was not collected for 13 minutes, but by that time there was a significant concentration of the tracer in the urine.

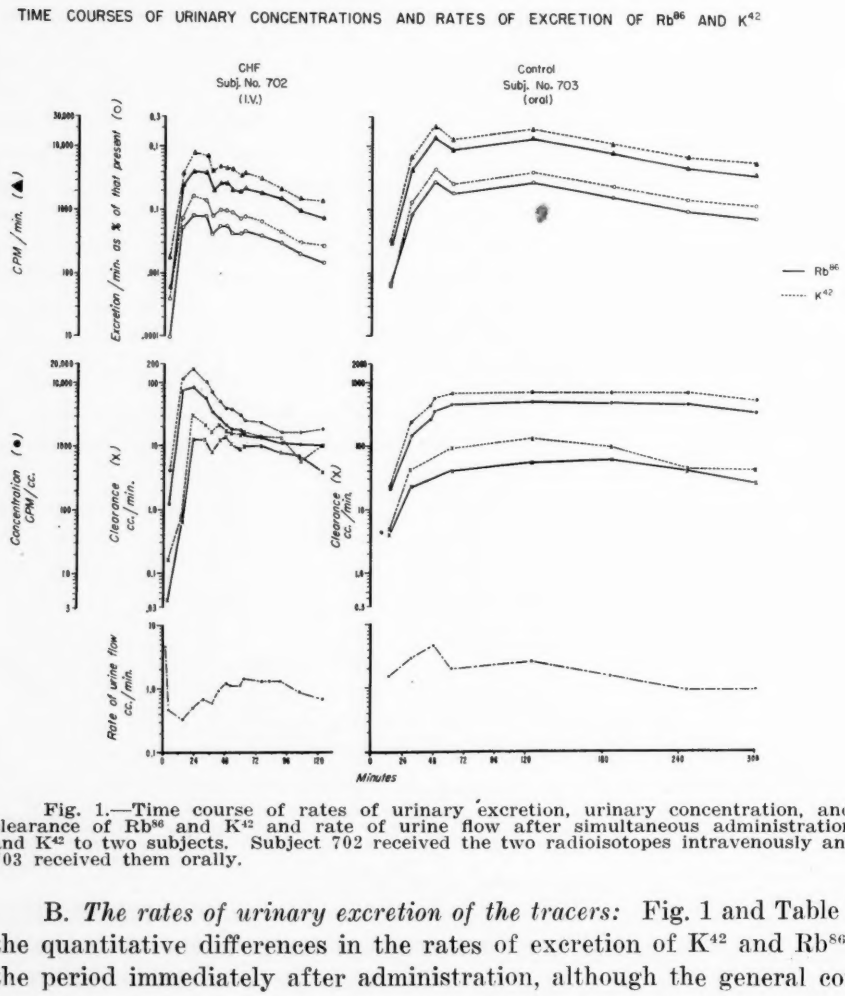



Fig. 1.—Time course of rates of urinary excretion, urinary concentration, and urinary clearance of $Rb^{86}$ and $K^{42}$ and rate of urine flow after simultaneous administration of $Rb^{86}$ and $K^{42}$ to two subjects. Subject 702 received the two radioisotopes intravenously and Subject 703 received them orally.

B. *The rates of urinary excretion of the tracers:* Fig. 1 and Table II show the quantitative differences in the rates of excretion of $K^{42}$ and $Rb^{86}$ during the period immediately after administration, although the general configura-

tions of the time course curves are remarkably similar. This is true for concentration of $Rb^{86}$ and $K^{42}$ in the urine, for urinary clearance, and for excretion expressed as a percentage of that remaining in the body. In the subject who received the isotope intravenously (Subject 702) the maximal values of these parameters were achieved in 22 minutes, whereas 48 minutes were required to reach maximal values after oral administration (Subject 703). Although the concentration of the tracers in the urine reached a higher level in the

TABLE II. URINARY EXCRETION OF $Rb^{86}$ AND $K^{42}$—FIRST 120 MINUTES

| SUBJECT | DOSAGE | | APPEARANCE TIME | | RECOVERY | | | | TOTAL URINARY VOLUME (ML.) | AVERAGE RATE OF URINARY FLOW (ML./MIN.) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TOTAL CPM | | % INJ. DOSE | | | |
| | $Rb^{86}$ (CPM) | $K^{42}$ (CPM) | $Rb^{86}$ (MIN. : SEC.) | $K^{42}$ (MIN. : SEC.) | $Rb^{86}$ | $K^{42}$ | $Rb^{86}$ | $K^{42}$ | | |
| 702 | $5 \times 10^7$ | $5 \times 10^7$ | 0:50 to 3:55 | 0:50 to 3:55 | 245,194 | 428,926 | 0.44 | 0.85 | 113.4 | 0.92 |
| 703 | $5 \times 10^7$ | $5 \times 10^7$ | < 13:30 | < 13:30 | 1,303,747 | 1,912,324 | 2.61 | 3.82 | 336.0 | 2.71 |

subject who received the tracers intravenously, the subject who received the tracers orally had a greater mean rate of excretion which was several times more rapid and which was maintained for a longer period of time. In the subject who received the tracers orally, the rate of urinary flow was approximately three times as great as that in the subject who received the tracers intravenously. Subject 702 showed the same discordant relationship between urinary volume and urinary concentration of the tracers which was noted previously for $Cl^{36}$ (Reference 3) and $Hg^{203}$ (Reference 7). Figs. 1 and 2 show a significant relationship between the urinary excretion of $Rb^{86}$ and $K^{42}$ although at all times $K^{42}$ was excreted more rapidly than $Rb^{86}$. The average ratio of the excretion of $Rb^{86}$ to $K^{42}$ was 0.52 and 0.68 in Subjects 702 and 703, respectively.

The specific activity of $K^{42}$ in the plasma and urine as well as the "specific activity" of $Rb^{86}$ ($Rb^{86}/K^{39}$) are shown in Fig. 3. In both subjects the specific activity of $K^{42}$ was higher in urine than in plasma and the specific activity of $K^{42}$ in the urine was greater than that of $Rb^{86}$. The relationship of the specific activities of $Rb^{86}$ and $K^{42}$ in the plasma was variable. The specific activities in both plasma and urine following intravenous administration of the tracers were greater and declined more rapidly than those following oral administration.

*Comment:* Although qualitatively the patterns of excretion showed a fairly constant relationship, the quantitative differences demonstrated that $Rb^{86}$ did not reflect precisely potassium excretion; potassium was excreted almost twice as rapidly as $Rb^{86}$. This was illustrated in the excretion as a percentage of that present in the body as well as in the urinary concentration, urinary clearance (Fig. 1), and specific activity (Fig. 3). There were individual differences between the two subjects. Differences in the same subject might be expected under varying conditions of potassium intake and metabolism when observed for prolonged periods of time. Such observations will be presented later in this report. Even though the concentrations of $Rb^{86}$ and

$K^{42}$ in the plasma were variable but approximately equal, the increased excretion of $K^{42}$ as compared to $Rb^{86}$ indicated a more rapid and possibly a preferential excretion of potassium. It is interesting that $Rb^{86}$ and $K^{42}$ should be transferred from the plasma at essentially indistinguishable rates,[1] while at the same time the rates of transfer of the two isotopes across the renal parenchyma definitely differed.

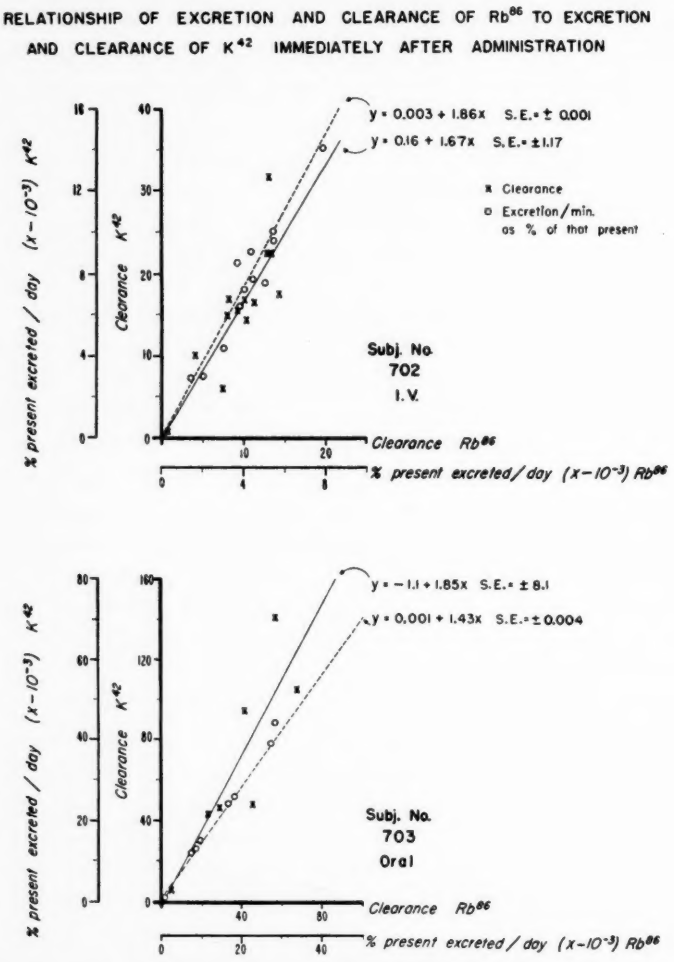

RELATIONSHIP OF EXCRETION AND CLEARANCE OF Rb86 TO EXCRETION AND CLEARANCE OF K42 IMMEDIATELY AFTER ADMINISTRATION
y = 0.003 + 1.86x S.E. = ± 0.001
y = 0.16 + 1.67x S.E. = ± 1.17
x Clearance
o Excretion/min. as % of that present
Subj. No. 702 I.V.
Clearance K42
% present excreted / day (x−10⁻³) K42
Clearance Rb86
% present excreted / day (x−10⁻³) Rb86
y = −1.1 + 1.85x S.E. = ± 8.1
y = 0.001 + 1.43x S.E. = ± 0.004
Subj. No. 703 Oral

Fig. 2.—A plot of excretion data for $K^{42}$ and $Rb^{86}$ showing the relationship of these two tracers with respect to each other expressed as the percentage of tracer remaining in the body and as the urinary clearance of the tracer. The equations for the respective regression curves are indicated for the first 120 minutes.

The excretion of both isotopes manifested the phenomenon of "skimming," i.e., excretion of the isotope before its entry into the major cellular compartments. That the degree of skimming was greater following administration of the tracer by vein than by the oral route was demonstrated by the higher specific activity and by the greater difference in specific activities in plasma and urine of Subject 702 than of Subject 703. Although Subject 703 received more carrier potassium with the tracers than did Subject 702, the amount of

potassium given (less than 2 Gm.) would not cause differences of the order shown by Figs. 1, 2, and 3. The reason for the extremely rapid rate of excretion by Subject 703 is not apparent, but it was probably related to the fact that the tracer was administered at a time when potassium was being "shifted" out of the body. Had she continued at this rate of excretion, 31 per cent of the $Rb^{86}$ and 45 per cent of the $K^{42}$ ingested would have been eliminated in 24 hours. Large amounts of potassium excreted with these tracers accounted for the lower specific activity in the urine. The increase in urinary volume in this patient, which was approximately three times that for Subject 702, was not totally responsible for the differences in excretion of $Rb^{86}$ and $K^{42}$ since it was five to six times that of Subject 702.

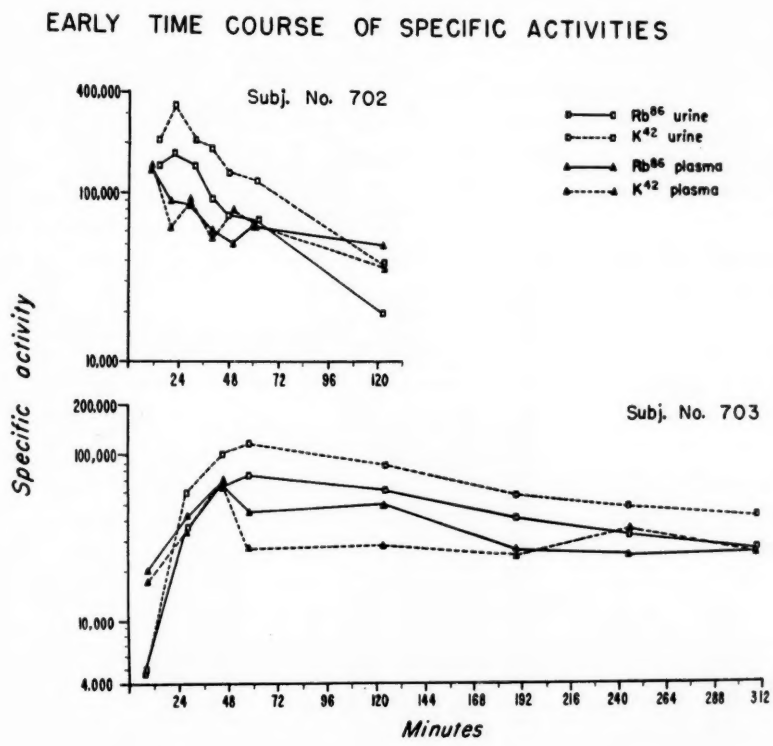



Fig. 3.

In these subjects, as in those observed with other tracer substances, the absolute values for clearance were unreliable during periods of rapid changes in concentration of the tracers in the blood.

2. *Recovery of the Isotope by Various Routes.*—The four subjects were not observed for the same length of time since the counts in the urine and plasma approached background levels too low for counting at different times and since one of the subjects expired at the end of the fortieth day of observation. Therefore, the first 30-day period of study was selected for comparison of the recovery of $Rb^{86}$ for all subjects.

| SUBJECT | INJECTED DOSAGE (CPM) | 30-DAY RECOVERY: ALL ROUTES % OF INJECTED DOSAGE | URINARY % OF INJECTED DOSAGE | URINARY % OF RECOVERED | FECAL % OF INJECTED DOSAGE | FECAL % OF RECOVERED | OTHER % OF INJECTED DOSAGE | OTHER % OF RECOVERED |
|---|---|---|---|---|---|---|---|---|
| 601 | $2 \times 10^8$ | 15.2 | 11.7 | 76.9 | 3.5 | 23.1 | 0 | 0 |
| 602 | $2 \times 10^8$ | 40.6 | 31.5 | 77.7 | 8.9 | 21.8 | 0.2* | 0.4 |
| 603 | $2 \times 10^8$ | 35.1 | 25.1 | 71.7 | 9.9 | 28.2 | 0.1* | 0.2 |
| 604 | $2 \times 10^8$ | 44.3 | 17.4 | 39.3 | 20.7 | 46.6 | 6.2* | 14.0 |

*Sputum.

Table III and Fig. 4, *A* show, as a percentage of the injected dose, the $Rb^{86}$ which had been recovered by all routes studied by the end of the thirtieth day. A total of 15.2 and 40.6 per cent had been recovered, 11.7 and 31.5 per cent in the urine, and 3.5 and 8.9 per cent in the stool, for control Subjects 601 and 602, respectively. A total of 35.1 and 44.3 per cent was recovered, 25.1

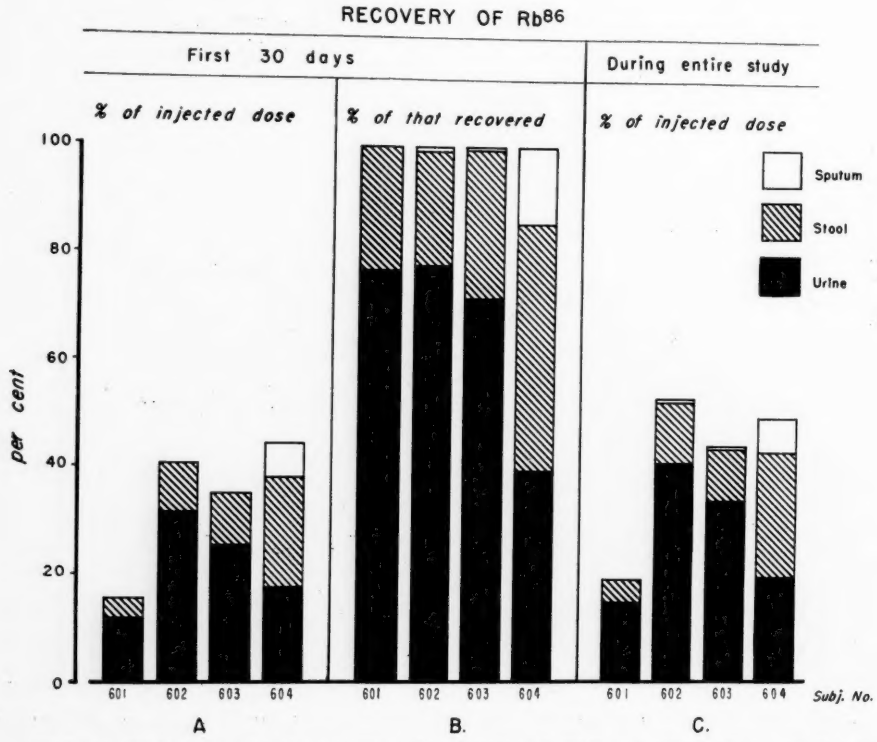



Fig. 4.—Histogram showing routes of recovery of $Rb^{86}$. *A*, Percentage of the injected dose recovered during the first 30 days of the study. *B*, Recovery of $Rb^{86}$ expressed as percentage of the total amount recovered in the first 30 days of the study. *C*, Percentage of the injected dose of $Rb^{86}$ recovered during the entire period of study.

and 17.4 per cent in the urine, and 9.9 and 20.7 per cent in the stool, for Subjects 603 and 604 with congestive heart failure. Subject 604 with severe congestive heart failure, from whom the greatest recovery was made, also

TOTAL EXPERIMENTAL RECOVERY

| | ALL ROUTES | URINARY | | FECAL | | OTHER | |
|---|---|---|---|---|---|---|---|
| ATION OF STUDY DAYS) | % OF INJECTED DOSAGE | % OF INJECTED DOSAGE | % OF RECOVERED | % OF INJECTED DOSAGE | % OF RECOVERED | % OF INJECTED DOSAGE | % OF RECOVERED |
| 38 | 18.9 | 14.8 | 78.3 | 4.1 | 21.6 | 0 | 0 |
| 44 | 53.0 | 41.2 | 77.7 | 11.6 | 22.0 | 0.2* | 0.3* |
| 48 | 44.2 | 33.4 | 75.7 | 10.7 | 24.3 | 0.1* | 0.2* |
| 40 | 49.7 | 19.4 | 39.1 | 23.4 | 47.1 | 6.9* | 13.8* |

excreted 6.2 per cent in sputum. Fig. 4, *B* presents the recovery of $Rb^{86}$ by various routes as a percentage of that recovered by the end of 30 days.

The total recovery at the end of the study period of each subject is also presented in Table III and Fig. 4, *C*. For all subjects, except the one who expired, the duration of study represented, approximately, the time required for the counts in plasma and urine to reach background levels. For the two control subjects, 18.9 and 53.0 per cent of the injected dose were recovered, and for the two subjects with congestive heart failure, 44.2 and 49.7 per cent were recovered. The partition into urinary, fecal, and other routes of recovery is shown in Table III and Fig. 4.

*Comment:* There was no significant difference in the recovery of $Rb^{86}$ between the two subjects with congestive heart failure and control Subject 602, who were studied simultaneously under the same metabolic conditions. However, control Subject 601 excreted less than one-half as much as any of the other subjects. The reason for the low percentage recovery in this patient is not apparent but might have been due, in part, but not entirely, to a different dietary intake. Even though this patient had slower excretory rates of $Rb^{86}$ the percentage of the total amount recovered which was excreted by way of the urine and stools was the same as for Subjects 602 and 603. It is interesting to note that the loss of $Rb^{86}$, and probably potassium, by way of the stools was from 20 to 25 per cent of the total excretion from the body. This is in contrast to the low fecal excretion of $Cl^{36}$, which was from 1 to 11 per cent of total recovery.[3] Subject 604, who had persistent diarrhea, had an even larger fecal loss of $Rb^{86}$. This was 47 per cent and exceeded the 39 per cent urinary excretion. In this subject, who insisted on chewing tobacco and who suffered from chronic suppurative bronchitis, the recovery of $Rb^{86}$ in the sputum was approximately 14 per cent of the total recovery. The recovery of $Rb^{86}$ from the sputum of the other subjects was negligible.

The duration of these experiments (approximately 40 to 50 days) was determined by the time required for the radioactivity to reach levels too low for accurate counting. This was considerably shorter than the duration of the experiments on subjects who received $Na^{22}$ (Reference 8) and $Cl^{36}$ (Reference 3), even though the amount of $Rb^{86}$ recovered was only 19 to 53 per cent of the injected dose. These differences were due to the fact that the *major* compartment of $Rb^{86}$ was intracellular whereas for $Na^{22}$ and $Cl^{36}$ the *major* compartment was extracellular. Once having entered the intracellular

compartment, there was a lag in return to the extracellular one, and since excretion was from the extracellular compartment, the $Rb^{86}$ in the plasma and urine reached low levels with a relatively large percentage of the injected dose still remaining in the large cellular mass of the body. It was demonstrated for these same subjects that the concentration of $Rb^{86}$ in the erythrocytes was still relatively high while the content of $Rb^{86}$ in the plasma and urine was very low.[1] This lag in return of $Rb^{86}$ to the extracellular compartment has been shown to exist for various organs.[9] Because of such lags,

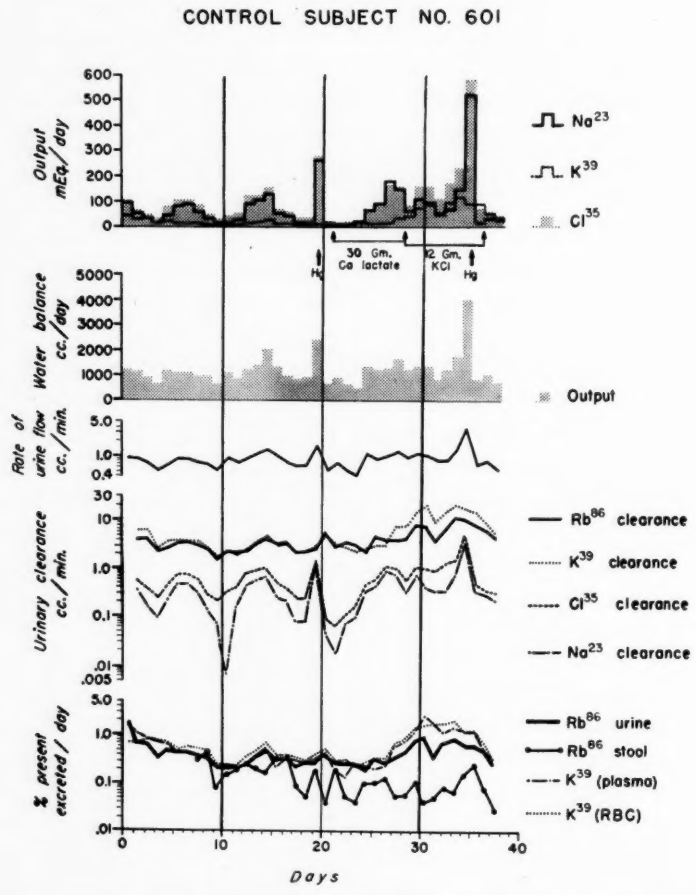



Fig. 5.—Simultaneous time course curves of various parameters for Subject 601, including the influence of drugs.

dietary intake would be expected to have less effect on intracellular elements such as $Rb^{86}$ and potassium than on elements such as sodium and chloride, for which the dietary intake enters the major compartment directly. Because of the lags in return of $Rb^{86}$ (and potassium) to the extracellular compartment, it was not possible in these experiments to exert so precise a control over the removal of $Rb^{86}$ from the body as was possible with $Na^{22}$ and $Cl^{36}$. This is important in considerations of radiation effects consequent to internal contamination by $Rb^{86}$ and $Cs^{134}$ (Reference 10).

3. *Daily Excretion and Clearance of $Rb^{86}$ and Balance of Potassium, Chloride, and Sodium.*—The data concerned with daily excretion of $Rb^{86}$, potassium, chloride, sodium, and water are summarized in Figs. 5, 6, 7, and 8 in a manner so as to permit correlation between the various factors which may influence excretion. In each figure are shown the average daily urine flow, the average daily clearances of radiorubidium, potassium, chloride, and sodium, the total daily urinary excretion of radiorubidium and potassium, and the

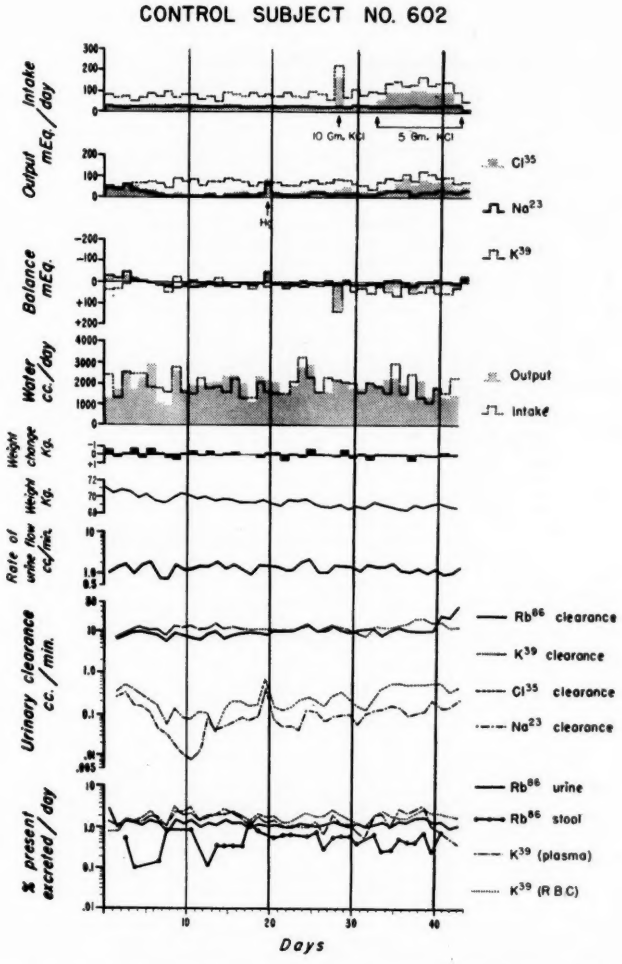



Fig. 6.—Simultaneous time course curves of various parameters for Subject 602, including balance data and the influence of drugs.

daily fecal excretion of radiorubidium as a percentage of that present in the body as well as the daily intake, output, and balance of potassium, chloride, sodium, and water, and the body weight and change in body weight. Various drugs and procedures employed during the study are also indicated. For Subject 601, Fig. 5, although the intake of the electrolytes was kept constant,

the precise quantities were not known and therefore intake and balance are not indicated. It should also be noted that the daily excretion of potassium is expressed as a percentage of that in the body estimated from the concentration of radiorubidium and potassium in both the plasma and erythrocyte compartments.[2]

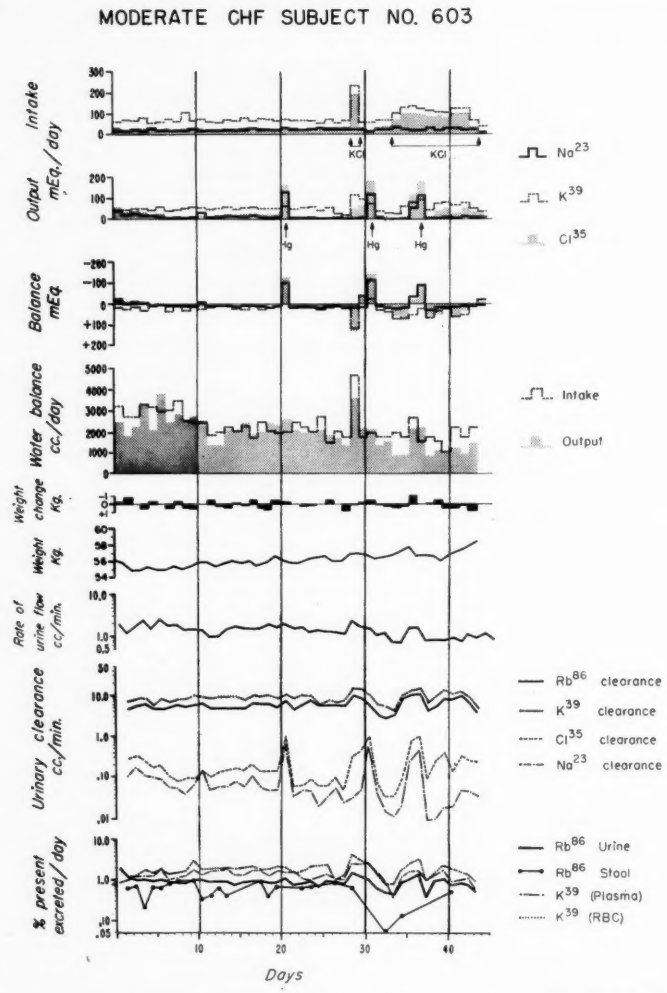



Fig. 7.—Simultaneous time course curves of various parameters for Subject 603, including balance data and the influence of drugs.

The daily output of $Rb^{86}$ is presented as a percentage of that present in the body which was excreted each day to enable a comparison of the excretory phenomena at the later periods in the study when the counts approached background and actual counts expressed as a percentage of the injected dose would produce a distorted picture of excretory function. The data presented in this fashion are comparable throughout the study and also permit comparison with the excretion of $K^{89}$ expressed in the same manner. These methods

of calculation have been presented previously[3] and may be made for any route or routes of excretion or collection of body fluids and electrolytes.

It may be noted in Figs. 5 through 8 that certain general variations in the excretory patterns were similar. This was especially true for sodium, chloride, and water in all subjects and for the variations in excretion expressed as a percentage of that present in the body and clearances of $Rb^{86}$ and potassium. Whereas this was also true for the comparison of $Rb^{86}$ and potassium with sodium, chloride, and water in the subjects with congestive heart failure, there were greater deviations in the excretion of $Rb^{86}$ and potassium from the pattern of excretion of sodium, chloride, and water in the control subjects. These differences and the differences between the excretion of $Rb^{86}$ and potassium in specific instances will be discussed in the sections to follow.

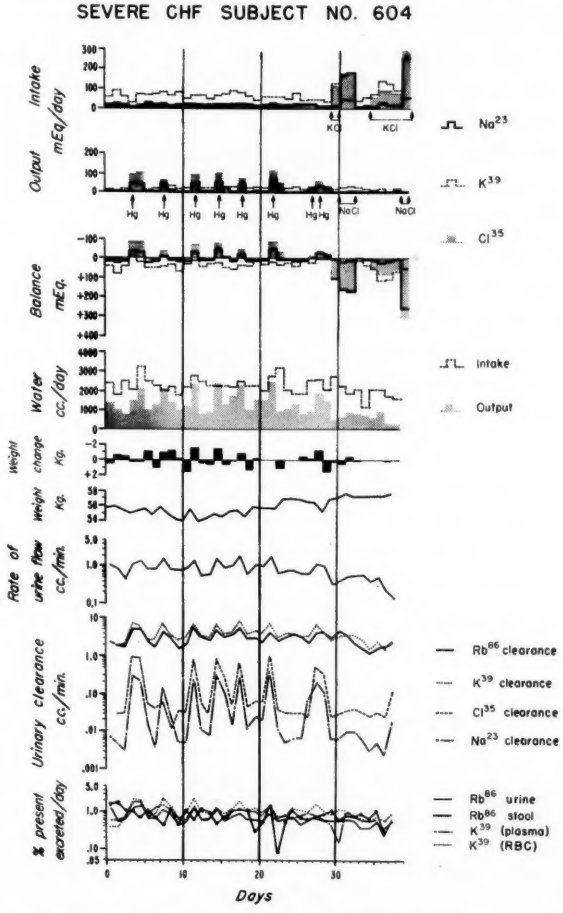



Fig. 8.—Simultaneous time course curves of various parameters for Subject 604, including balance data and the influence of drugs.

A. *Excretion of $Rb^{86}$ and potassium as a percentage of that present in the body:* As illustrated by Figs. 5 through 8, the daily rate of excretion of potassium in the four subjects ranged from about 0.5 to 2.0 per cent of that present in the body. The rate of urinary excretion of $Rb^{86}$ expressed as a

percentage of that present in the body was usually less than that for potassium estimated from the concentration of $Rb^{86}$ and potassium in the plasma and erythrocyte compartments except for Subject 601 (Fig. 5). The excretion of potassium as a percentage of that in the body estimated from the concentrations of $Rb^{86}$ and potassium in the erythrocyte compartment was usually higher in all subjects than that based on the concentrations of $Rb^{86}$ and potassium in the plasma. The excretion of both $Rb^{86}$ and potassium as a percentage of that present in the body was most in control Subject 602, followed by Subjects 603 and 604 with congestive heart failure and control Subject 601, in that order.

Increases in the dietary intake of potassium as potassium chloride resulted in variable effects among the four subjects. Subject 604, who was extremely ill with congestive heart failure at the time of administration of potassium chloride, had no increase in excretion of $Rb^{86}$ and potassium, whereas Subject 603 with moderately severe congestive heart failure had a definite increase in excretion of $Rb^{86}$ and potassium on two occasions when potassium chloride was administered. When 12 Gm. of potassium chloride was added to the daily dietary intake of control Subject 601, she also showed a definite increase in the excretory rate of potassium and $Rb^{86}$. The other control subject (Subject 602) showed only a slight increase in the excretion of these elements when the intake of potassium was increased.

The effects of a mercurial diuretic (Mercuhydrin) on the excretion of $Rb^{86}$ are summarized in Figs. 5 through 8 and Table IV. The control Subjects 601 and 602 excreted less $Rb^{86}$ on the day that the diuretic was administered than during the preceding days and the following day. Subjects 603 and 604 with congestive heart failure had more rapid excretion of $Rb^{86}$ on the day the diuretic was administered than during previous control days and the day subsequent to diuresis. When the net excretion on the day of and the day after the administration of the mercurial diuretic was considered, there was no net increase in excretion of $Rb^{86}$ as compared to excretory rates during control intervals except in Subject 604, who showed a distinct increase (Table IV).

TABLE IV

| SUBJECT | AVERAGE DAILY EXCRETION (% PRESENT) | AVERAGE DAILY EXCRETION ON HG (% PRESENT) | AVERAGE DAILY EXCRETION ON DAY AFTER HG (% PRESENT) | RATIO HG DAY : CONTROL DAY | RATIO DAY AFTER HG DAY : CONTROL DAY | RATIO AVERAGE DAILY EXCRETION OF HG DAY AND DAY AFTER : CONTROL DAY |
|---|---|---|---|---|---|---|
| | | | *Low K Intake* | | | |
| 601 | 0.44 | 0.26 | 0.38 | 0.59 | 0.86 | 0.73 |
| 602 | 1.30 | 1.11 | 1.31 | 0.85 | 1.01 | 0.93 |
| 603 | 0.95 | 1.21 | 0.77 | 1.27 | 0.81 | 1.04 |
| 604 | 0.58 | 1.02 | 0.81 | 1.76 | 1.40 | 1.58 |
| | | | *High K Intake* | | | |
| 601 | 0.61 | 0.62 | 0.60 | 1.02 | 0.98 | 1.00 |
| 603 | 0.88 | 1.48 | 0.51 | 1.68 | 0.60 | 1.14 |

In Figs. 5, 6, and 7, depicting the urinary responses of the two control subjects and the subject with moderate congestive heart failure, the increased excretion of sodium and chloride was not accompanied by an increased excretion of $Rb^{86}$ and potassium. In fact, excretion of $Rb^{86}$ and potassium on some occasions in these three subjects decreased in response to the mercurial diuretic. In Subject 604 (Fig. 8), with severe congestive heart failure, each administration of the mercurial diuretic resulted in a marked increase in the excretion of sodium and chloride and a less marked but definite increase in the excretion of $Rb^{86}$ and potassium. There were variations in the rates of excretion of $Rb^{86}$ and potassium in response to mercurial diuretics among subjects and from time to time in the same subject.

The fecal excretion of $Rb^{86}$ in the four subjects is presented in Table III and Figs. 5 through 8. In every subject there were some days during which the fecal excretion of $Rb^{86}$ as a percentage of the $Rb^{86}$ remaining in the body exceeded the urinary excretion. This occurred most often for the subject with severe congestive heart failure and diarrhea (Fig. 8). For the two control subjects and for Subject 603 with moderate congestive heart failure, the fecal excretion was about one-third of the urinary excretion for the entire period of study while for the subject with severe congestive heart failure and diarrhea, fecal excretion of $Rb^{86}$ exceeded that in the urine. For this subject, excretion in sputum was greater than one-third of that in the urine. The variations in fecal excretion were greater than the variations in the urinary excretion and did not appear to be related to the daily fluctuations in urinary excretion. These fluctuations in the fecal excretion could have been due to the nature of the stool, kind of food in the diet, as well as other factors. Mercurial diuretics and changes in the dietary intake of potassium had no apparent effect. The daily excretion of $Rb^{86}$ in the stool ranged from 0.03 to 2.0 per cent of that present in the body or from 21 to 47 per cent of the total excretion.

*Comment:* Even if $Rb^{86}$ traced potassium as well as $Cl^{36}$ traced $Cl^{35}$, some differences in excretion as a percentage of that calculated to be present in the body would be expected.[3, 11] In fact, since $Rb^{86}$ and $K^{39}$ are primarily intracellular elements with relatively slow rates of exchange, such differences would be magnified. Excretion of electrolytes into the urine is primarily from the extracellular compartment, which for $Rb^{86}$ and potassium is a *minor* compartment and for sodium and chloride the *major* compartment. Because of the large intracellular mass of potassium, exchange is slow and incomplete and excretion of potassium may be disproportionately made up of recent dietary intake which has not equilibrated with that in the body. That this was also true for $Rb^{86}$ was manifested by the high excretory rates on the first day or so following injection. However, once having entered into the cellular compartment, transfer back into the plasma may lag sufficiently to be bypassed by recently ingested potassium which would result in the slower excretory rate of $Rb^{86}$ shown in Figs. 5 through 8.

There is an important difference between the expression of excretion of $Rb^{86}$ as a percentage of that remaining in the body determined *directly* and the excretion of potassium expressed as a percentage of that in the body which was *calculated* from space of $Rb^{86}$ and the concentration of potassium in either plasma or erythrocytes. Such calculations assume that complete equilibration of the tracer existed and further that $Rb^{86}$ was an adequate tracer of potassium. The limitations and possible errors of such an approach have been discussed in detail previously.[1, 2] Since the mass of potassium as estimated from a study of the plasma was greater than the mass of potassium as estimated from a study of the erythrocytes, the excretion of potassium as a percentage of that present in the body was less when plasma values were used.

Even though these observations were conducted over long periods of time, the problem of equilibrium of distribution of the tracer was present at all times in as much as the entrance of the tracer into the cellular compartment was slow and, likewise, the return of the tracer to the plasma compartment lagged behind excretion. With multiple compartments of different exchange rates, variations in dietary intake of potassium, and changes in kaliokinetic phenomena in diseased states, a constant relationship of $Rb^{86}$ to potassium would not be expected. Over short periods of time (several hours) where such factors could not be manifested in urinary excretory rates, a fairly constant relationship of $Rb^{86}$ to potassium might be expected. In fact, simultaneous administration of $K^{42}$ and $Rb^{86}$, presented earlier in this paper, showed this to be the case. Although the simultaneous administration of $K^{42}$ and $Rb^{86}$ showed that for short intervals of time the factors just presented were of little or no significance, there was still a quantitative difference in the rates of excretion of $Rb^{86}$ and potassium.

Loss of significant amounts of potassium by way of the feces is well known and these observations revealed that $Rb^{86}$ was likewise lost in significant amounts in the stools, the amount varying from 21 to 47 per cent of the total amount of $Rb^{86}$ excreted.

B. *Urinary clearance of $Rb^{86}$, $K^{39}$, sodium, and chloride:* Figs. 5 through 8 present the urinary clearance rates for $Rb^{86}$, $K^{39}$, sodium, and chloride. Subject 601 had the highest clearance rates for sodium and chloride, control Subject 602 only slightly greater or about the same as Subject 603 with moderate congestive heart failure, and Subject 604 with severe congestive heart failure the lowest rates. It is interesting to note, however, that control Subject 601, with the highest sodium and chloride clearances, manifested the lowest rates of clearance of $Rb^{86}$ and $K^{39}$, which were about the same as for Subject 604 with severe failure. Control Subject 602 showed the highest rates of clearance of potassium and $Rb^{86}$, which were somewhat higher than those for Subject 603 with moderate failure. Subjects 602 and 603, who had the highest rates of clearance of potassium and $Rb^{86}$, also had the highest rates of urinary flow. This relationship did not exist between clearances of sodium and chloride and rates of urinary flow with the exception of the days of mercurial diuresis. When a mercurial diuretic was administered, all subjects showed an increase in clearances of sodium and chloride, whereas only

the subject with severe congestive heart failure consistently and distinctly demonstrated this increase for $Rb^{86}$ and $K^{39}$. Considerable variations in the relationship of individual diuretic responses are apparent in the figures.

RELATION OF DAILY CLEARANCES OF $Rb^{86}$ TO CLEARANCE OF $K^{39}$ (cc./min.)

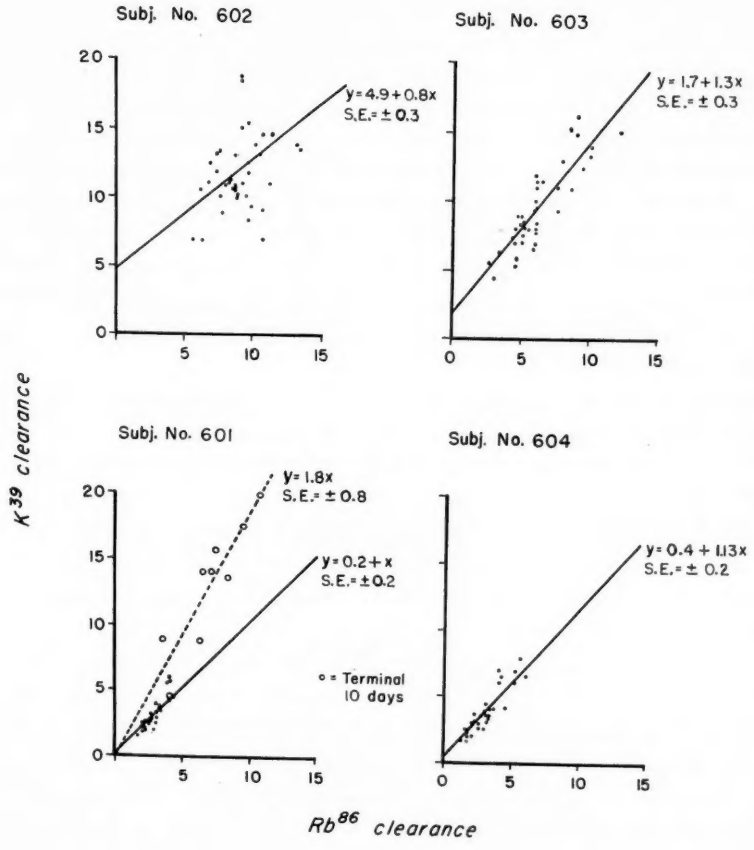



Fig. 9.

For the most part throughout the studies, clearance of $Rb^{86}$ was lower than clearance of potassium. The relationship of clearance of $Rb^{86}$ to that of potassium is shown in Fig. 9 as well as in Figs. 5 through 8. Control Subject 601 demonstrated a close relationship between clearance of $Rb^{86}$ and of potassium for the first twenty-seven days of the study, and control Subject 602 showed a close relationship from the twenty-fourth to the thirtieth day. The two subjects with congestive failure demonstrated a good qualitative relationship, but only on one or two occasions did they have the same quantitative values for clearance of $Rb^{86}$ and potassium. For the two control subjects, when clearance of potassium increased, clearance of $Rb^{86}$ did not increase

proportionately. This is shown from the twenty-seventh to the thirty-fifth day for Subject 601 and from the thirty-second to thirty-ninth days for Subject 602. Near the end of the studies the concentrations of $Rb^{86}$ in plasma and urine were too low for accurate determination of clearance. The qualitative and quantitative relationships of the clearances of $Rb^{86}$ and potassium are shown in Figs. 5 through 9.

*Comment:* The urinary clearances of $Rb^{86}$ and potassium showed a variable difference. At times the values of the two were quantitatively the same and at other times they varied discordantly. By simultaneous injection of $Rb^{86}$ and $K^{42}$ a rather constant difference in the excretion of these two radioisotopes was demonstrated and the greater clearance rate of potassium observed here was expected. In addition to the difference in renal excretion, other factors were operating in as much as variations in relationship of these rates of clearance occurred. Such factors as skimming and lags in cellular compartment exchange are important. The clearances shown are daily average clearances calculated from single daily values for the plasma. Failure to relate diurnal variations in urinary excretion to the concentration of the substance in the blood at the time of greatest excretion could have produced some of the variations shown here.

C. *Specific activity:* "Specific activity" as used in this sense is not a true specific activity but rather a ratio of the concentration of $Rb^{86}$ to that of $K^{39}$ since $Rb^{85}$ was not measured chemically. However, this ratio is useful in testing $Rb^{86}$ as a tracer of potassium, but errors in interpretation may arise from the use of this ratio if it is used as a true potassium or rubidium specific activity. Fig. 10 shows the "specific activity" of $Rb^{86}$ in the erythrocytes, plasma, and urine as well as urinary clearances of $Rb^{86}$ and potassium and the possible influence of drugs.

Throughout the study the specific activity in the erythrocytes was usually higher than that in the plasma and the specific activity in the urine was usually lower than that in the plasma except for the first few hours of study. This ratio of $Rb^{86}$ to potassium in the erythrocytes required three days to rise to a point equal to or greater than that in the plasma. The specific activity in the erythrocytes continued to rise to a plateau which was maintained for from seven to seventeen days while at the same time the specific activity in the plasma and urine continued to fall. All of the curves for the four subjects showed considerable variations from day to day and especially in the last two weeks of observation, when the concentration of $Rb^{86}$ in the urine and plasma had declined to a level too low for accurate counting. In spite of the day-to-day variations in these ratios certain patterns were apparent (Fig. 10). For example, in control Subject 601 the specific activity in the plasma was almost the same as that in the urine, whereas in control Subject 602 the specific activity in plasma was more nearly equal to that in the erythrocytes for the first seventen days. In the two subjects with congestive heart failure (Subjects 603 and 604) the values of specific activity in the plasma tended to be intermediate between those of the erythrocytes and those of the urine.

When urinary excretion of potassium increased because of administration of potassium chloride or mercurial diuresis, the specific activity in the urine decreased (Fig. 10). The ratio of $Rb^{86}$ to potassium in the urine and plasma varied concordantly and discordantly.

*Comment:* The relatively slow rise in the specific activity of $Rb^{86}$ in the erythrocytes was indicative of a slow rate of exchange in this compartment[12] which accounted for the higher specific activity in the erythrocytes than in the plasma and urine. The reasons for the difference in general patterns of the parameters among the four subjects are unknown.

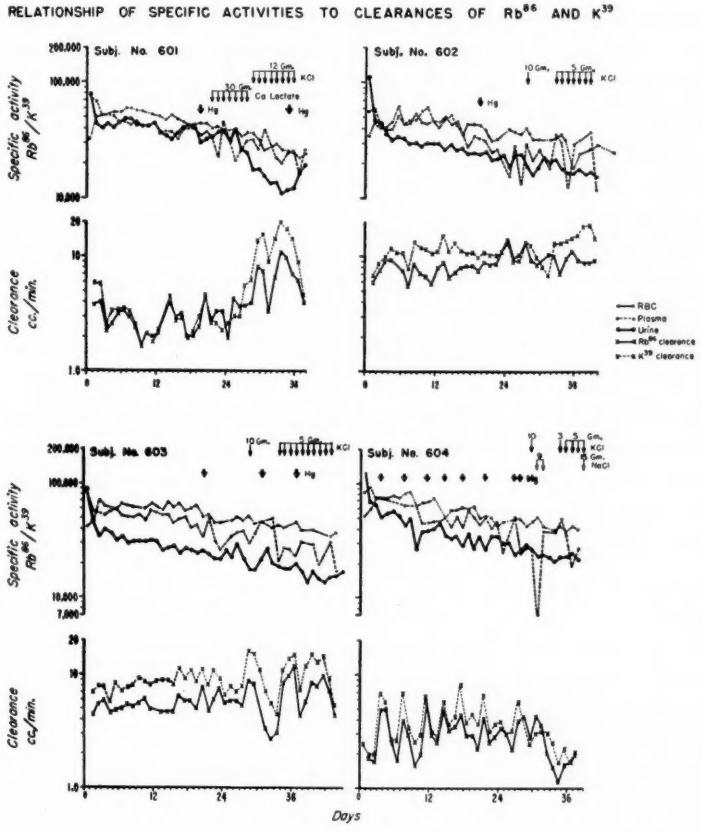



Fig. 10.

The rather large daily variations in the specific activity in the plasma during the last two weeks of observation probably resulted from errors in counting due to the low concentration of $Rb^{86}$ remaining in the plasma. However, there were daily variations which were not attributable to either counting error or change in the concentration of potassium in the urine. With such a small compartment as the extracellular fluid in contact with the large intracellular compartment, a small change in exchange rates between these compartments could result in daily fluctuations in the specific activity in the

plasma such as were observed in these experiments. Skimming phenomena during periods of increased intake of potassium or the effects of by-passing the major compartments by ingested potassium could result in a more rapid decline in specific activity in the urine as compared to that in the erythrocytes.

If $Rb^{86}$ traced potassium perfectly or with a constant ratio, when clearance increased, specific activity in the urine would fall only in relation to the biologic decay rates. Sudden fluctuations, greater than those of the biologic decay rates, would indicate the existence of lags in exchange, skimming, or changes in kaliokinetic or rubidiokinetic phenomena.

D. *The relationship of balance of potassium to that of sodium and chloride:* These experiments were designed to measure balance of potassium, sodium, and chloride, exclusive of extraurinary loss. The intake of these substances was not known precisely in control Subject 601 (Fig. 5), but measurements of balance were obtained in the other three subjects (Figs. 6, 7, and 8). It is apparent that all three subjects were in a positive potassium balance most of the time. This positive balance was of small magnitude in Subjects 602 and 603, but in Subject 604, who had severe congestive failure and persistent diarrhea, this *apparent* positive balance was greater. The effect of mercurial diuresis on balance of potassium was variable, whereas balance of sodium and chloride became negative in all subjects each time the mercurial diuretic was administered. The greatest effect of diuresis on potassium balance was noted in Subject 604 (Fig. 8). The interrelationships of balance of potassium, sodium, and chloride to water intake, urinary excretion, and body weight may be noted by inspection of Figs. 6, 7, and 8.

*Comment:* The small daily positive balance of potassium obtained in these experiments resulted in a large cumulative positive balance of potassium even in subjects without diarrhea. If $Rb^{86}$ precisely traced potassium in the stool, the loss of potassium by this route would have been 22, 24, and 47 per cent of the potassium excreted for Subjects 602, 603, and 604, respectively, a loss considerably greater than that observed for chloride with $Cl^{36}$ (Reference 3).

Since the amount of potassium chloride added to the dietary intake of potassium did not appreciably affect urinary excretion in these three subjects, the balance became more positive. One would expect an increase in intake of potassium to result in a greater excretion if the subjects were not deficient in potassium from a relatively low intake preceding the administration of potassium chloride. Had the greater intake continued, excretion probably would have changed to restore balance. The relatively small gains in body weight in the two subjects with congestive failure were probably due to edema fluid and were unimportant in balance of potassium.

4. *The Influence of Various Drugs on the Excretion of $Rb^{86}$ and Potassium.*—The drugs employed in these experiments were calcium lactate, potassium chloride, and a mercurial diuretic. Control Subject 601 (Fig. 5) received 30 Gm. of calcium lactate daily for one week without any detectable effect on the excretion of $Rb^{86}$ or potassium. All four subjects received potassium chloride near the end of the experimental period. As discussed

earlier in this report, the excretory response varied considerably among the four patients. Unfortunately, at the time the intake of potassium was increased, the concentration of $Rb^{86}$ in the plasma and urine was too low for accurate counting, thus making it difficult to test the influence of intake of potassium upon $Rb^{86}$ as a tracer of potassium.

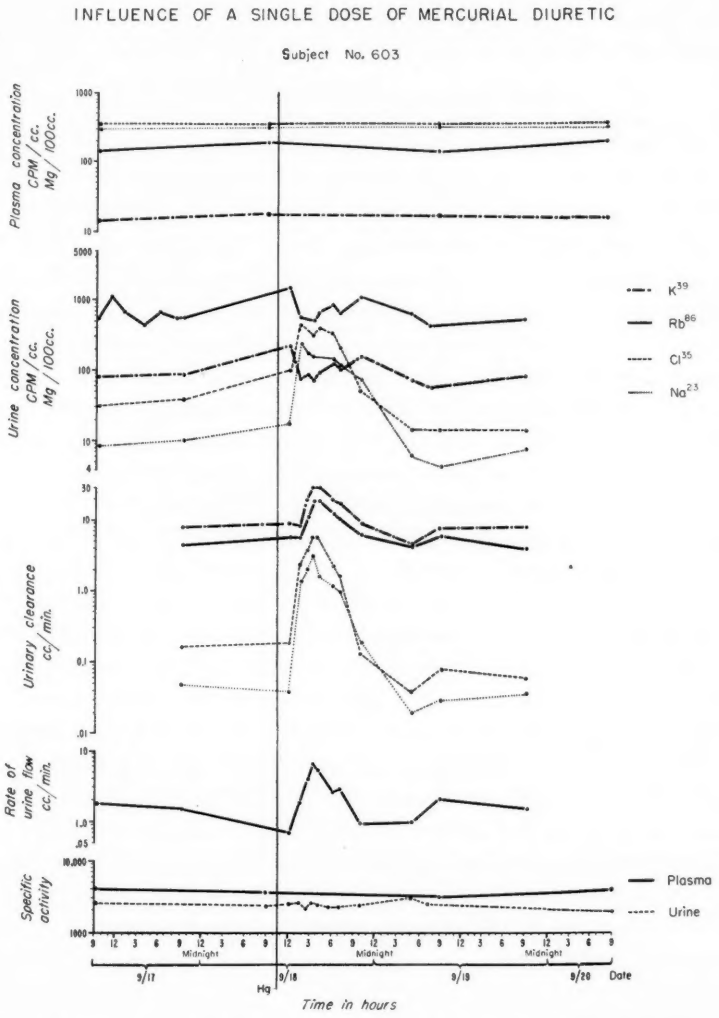



Fig. 11.—The influence of a single dose of a mercurial diuretic, Mercuhydrin, upon the electrolytes and tracers studied in the serum or plasma and urine of Subject 603.

The intravenous administration of a mercurial diuretic resulted in a similar excretory response in all subjects (Fig. 11, Subject 603). There was a greater increase in the excretion of sodium, chloride, and water than that for $Rb^{86}$ and potassium. The clearances of $Rb^{86}$ and potassium increased, but the urinary volume also increased disproportionately as to result in a decrease

in the concentrations of $Rb^{86}$ and potassium in the urine. The ratio of the concentration of $Rb^{86}$ to that of potassium in the urine as well as the ratio of the clearance of $Rb^{86}$ to that of potassium was remarkably constant.

*Comment:* The increased excretion of chloride, sodium, and water in response to mercurial diuretics is well known. The similarity of the curves of concentration of $Rb^{86}$ and potassium in the urine as well as those of clearance during a relatively short interval of increased excretion of potassium is further evidence that $Rb^{86}$ is at least a qualitative tracer of potassium.

## GENERAL DISCUSSION

In the studies described in this report the excretion of $Rb^{86}$ in the urine and stools and the excretion of potassium in the urine were measured. The greatest kaliokinetic and rubidiokinetic differences which have been observed were concerned with urinary excretion. These differences existed for short periods of study with $Rb^{86}$ and $K^{42}$ as well as over long periods of time for $Rb^{86}$ and potassium ($K^{39}$). These differences were largely quantitative rather than qualitative. Increased excretion of potassium was usually accompanied by increased excretion of $Rb^{86}$. There were some exceptions to this, most of which occurred in the latter part of the experiments when the radiometric assay of $Rb^{86}$ was inaccurate due to low levels of counts. The quantitative relationship of $Rb^{86}$ to potassium was not constant throughout these observations but constancy would not have been expected even if $Rb^{86}$ traced potassium precisely. The partition of potassium and $Rb^{86}$ into a large, slowly exchanging, cellular component and into a small extracellular component might be expected to result in differences in excretion of these substances since excretion of potassium could be disproportionately made up of recently ingested potassium which did not equilibrate with that already present in the body. Because of "skimming" and lags in exchange, differences in the excretion of $K^{42}$ and $K^{40}$ from that of $K^{39}$ would be expected, especially during periods of high intake of potassium. It is interesting to note that the rates of transfer of $Rb^{86}$ and $K^{42}$ across the vascular membrane showed no significant differences, yet at the same time the rates of transfer into the urine differed. This and the resultant differences in simultaneous specific activities of $Rb^{86}$ and $K^{42}$ in plasma and urine support differences in their renal excretory phenomena. Because such differences existed, it is apparent that $Rb^{86}$ is not a precise tracer of potassium, but observation of excretion of $Rb^{86}$ over prolonged periods provides valuable information concerning the behavior of the excretion of potassium.

Fecal excretion of $Rb^{86}$ conformed to previous knowledge of the excretion of potassium by this route. From 20 to 25 per cent of the $Rb^{86}$ recovered was excreted in the stools, and in one subject with diarrhea, the amount of $Rb^{86}$ recovered by this route was about 50 per cent (Table III). It is interesting that control Subject 601 had the lowest excretory rates of all subjects studied, yet the partition of $Rb^{86}$ which was excreted in the urine and stools was the same as for the other subjects without diarrhea.

Certain distinct differences between the excretory patterns of $Rb^{86}$ and potassium and those of sodium and chloride were apparent. The location in the body of the major mass or compartment of potassium and $Rb^{86}$ differed from that of sodium and chloride. With intake, nontracer materials would enter directly into the major compartment of sodium and chloride, whereas the minor compartment of potassium and $Rb^{86}$ would be entered directly and the major compartment only indirectly. Since excretion is from the minor compartment of $Rb^{86}$ and potassium, rubidiokinesis and kaliokinesis are not so greatly influenced by high dietary intake of nontracer potassium as are natriokinesis and chlorokinesis by intake of sodium and chloride. Such considerations are of importance in radiation injury in instances of internal radiocontamination with related elements.

## SUMMARY

Rubidiokinetic and kaliokinetic phenomena were studied in six subjects. In one control subject and one with congestive heart failure the phenomena were observed immediately following the simultaneous administration of $Rb^{86}$ and $K^{42}$ as tracers. Two control subjects and two subjects with congestive heart failure were followed continuously up to fifty days using $Rb^{86}$ only.

In the first two subjects, the tracers appeared in the urine collected by bladder catheters between 50 seconds and 4 minutes 55 seconds. Urinary concentration and clearance reached maximal levels in 23 minutes following intravenous administration and 48 minutes following oral administration. The urinary excretion of $Rb^{86}$ was 52 and 68 per cent that of $K^{42}$ in the two subjects, respectively, who received the tracers simultaneously. The rate of transfer across the vascular membrane was indistinguishable, however. Both isotopes manifested the phenomenon of skimming, i.e., excretion *without* entry into major cellular compartments.

Daily rates of excretion of potassium in four subjects observed for prolonged periods of time ranged from 0.5 to 2.0 per cent of that present in the body as estimated from calculations of space of $Rb^{86}$.

About 20 to 25 per cent of the tracer excreted was recovered in the stools with an increase to approximately 50 per cent in the presence of diarrhea.

Mercurial diuretics increased the urinary excretion of $Rb^{86}$ and potassium, but to a lesser degree than that of sodium and chloride.

Data are presented concerning daily balance measurements for sodium, potassium, chloride, and $Rb^{86}$ which permit a correlation of the excretory patterns of these elements.

These experiments indicate that $Rb^{86}$ is not a precise tracer of potassium, but because of its long half-life, it provided significant information about rubidiokinetic and kaliokinetic phenomena not obtainable by the use of $K^{42}$.

## REFERENCES

1. Burch, G. E., Threefoot, S. A., and Ray, C. T.: The Rate of Disappearance of $Rb^{86}$ From the Plasma, the Biologic Decay Rates of $Rb^{86}$ and the Applicability of $Rb^{86}$ as a Tracer of Potassium in Man With and Without Chronic Congestive Heart Failure, J. LAB. & CLIN. MED. **45**: 371, 1955.

2. Threefoot, S. A., Ray, C. T., and Burch, G. E.: Study of the Use of $Rb^{86}$ as a Tracer for the Measurement of $Rb^{86}$ and $K^{39}$ Space and Mass in Intact Man With and Without Congestive Heart Failure, J. LAB. & CLIN. MED. **45**: 395, 1955.
3. Ray, C. T., Threefoot, S. A., and Burch, G. E.: Studies on the Excretion of Chloride by Man With and Without Congestive Heart Failure, Using Long-Life Radiochloride, $Cl^{36}$, J. LAB. & CLIN. MED. **44**: 663, 1954.
4. Schales, O., and Schales, S.: A Simple and Accurate Method for the Determination of Chloride in Biologic Fluids, J. Biol. Chem. **140**: 879, 1941.
5. Burch, G., Reaser, P., Ray, T., and Threefoot, S.: A Method of Preparing Biologic Fluids for Counting of Radioelements, J. LAB. & CLIN. MED. **35**: 626, 1950.
6. Burch, G. E., Threefoot, S. A., and Reaser, P. B.: Some Aspects of Renal Excretion of $Na^{24}$ by Normal Subjects and by Patients With Congestive Heart Failure, Stanford M. Bull. **6**: 81, 1948.
7. Milnor, P., Burch, G., Ray, T., Threefoot, S., and Berenson, G.: Considerations of Renal, Hepatic, and Extremital Arteriovenous Differences in Concentration of Radiomercury of a Mercurial Diuretic, J. Clin. Invest. **29**: 72, 1950.
8. Threefoot, S., Burch, G., and Reaser, P.: The Biologic Decay Periods of Sodium in Normal Man, in Patients With Congestive Heart Failure and in Patients With the Nephrotic Syndrome as Determined by $Na^{22}$ as the Tracer, J. LAB. & CLIN. MED. **34**: 1, 1949.
9. Love, W. D., Romney, R. B., and Burch, G. E.: A Comparison of the Distribution of Potassium and Exchangeable Rubidium in the Organs of the Dog, Using $Rb^{86}$, Circulation Res. **2**: 112, 1954.
10. Threefoot, S. A., Burch, G. E., and Ray, C. T.: The Biologic Decay Rates and Excretion of Radiocesium, $Cs^{134}$, With Evaluation as a Tracer of Potassium in Dogs. J. LAB. & CLIN. MED. **45**: 313, 1955.
11. Burch, G. E., Ray, C. T., and Threefoot, S. A.: Some Theoretic Considerations of Electrolyte Space Measured by the Tracer Method in Intact Man, J. LAB. & CLIN. MED. **42**: 34, 1953.
12. Love, W. D., and Burch, G. E.: A Comparison of $Potassium^{42}$, $Rubidium^{86}$, and $Cesium^{134}$ as Tracers of Potassium in the Study of Cation Metabolism in Human Erythrocytes In Vitro, J. LAB. & CLIN. MED. **41**: 351, 1953.

# THE ANTIHYPERTENSIVE PROPERTIES OF SOME MERCAPTANS AND OTHER SULFUR-CONTAINING COMPOUNDS

HENRY A. SCHROEDER, M.D., EDNA M. MENHARD, B.S., AND
H. MITCHELL PERRY, JR., M.D.
ST. LOUIS, MO.

WITH THE TECHNICAL ASSISTANCE OF EVIE G. DENNIS

ROUTINE pharmacologic testing of vasoactive antihypertensive drugs has often been confined to the use of normotensive animals. If, however, hypertension, especially of the renal variety, is not an extension of the normal state, but is a new condition produced by vasoconstrictor substances not normally present, then the ideal agent for counteracting hypertension would be one which little affects normal blood pressure, but which specifically depresses elevated blood pressure. For the past several years in a search for such a substance a large number of compounds have been screened in this laboratory for prolonged antihypertensive properties in hypertensive animals.

In order to ascertain whether or not "specific" activity could be demonstrated a screening technique was developed which allowed rapid evaluation of any agent soluble in water as to its ability to block the action of norepinephrine or cause depression of blood pressure in rats with experimental renal hypertension. This report concerns aliphatic and aromatic sulfhydryl compounds and others with sulfur combined in the molecule.

## METHOD

Rats anesthetized with pentobarbital sodium, both normal (total 140) and made hypertensive by constriction of one renal artery (total 166), were connected to a Hamilton optical manometer or Sanborn electromanometer by a needle inserted into one femoral artery (in order to avoid disturbances of the carotid sinus) and a cannula placed in the opposite femoral vein for injection.[1] Tracheotomy was performed. Rats were considered to be hypertensive by an arbitrary diastolic level of 100 mm. Hg or more under anesthesia; the mean value was 119.0 mm. Hg, while that for normal rats was 75.3 mm. The rats weighed 220 to 270 grams, 80 per cent weighing close to 240 grams.

The response of the blood pressure to a standard dose of *l*-norepinephrine (0.3 $\gamma$) was observed, the substance to be tested injected, and the same dose of norepinephrine again given thirty minutes later. The blood pressure was recorded graphically before, during, and after injection of each compound and at intervals of five, ten, fifteen, twenty, and thirty minutes thereafter. A second and a third injection were subsequently given at half-hour intervals in order to discover maximal or cumulative effects of each substance; the pressor effect of norepinephrine was re-evaluated thirty minutes after each injection. Experiments usually lasted one to three hours and only sustained depressor responses were considered of interest. All compounds were neutralized to pH 7.4 before injection. No more than one compound was given to a rat; usually four or five of each type of rat were

From the Hypertension Division, Department of Internal Medicine, Washington University School of Medicine, and Barnes Hospital, St. Louis, Mo., under a grant-in-aid from the National Heart Institute, United States Public Health Service, and the Lasdon Foundation.

The authors are indebted to Monsanto Chemical Co., Eli Lilly and Co., Merck & Co., and Smith, Kline and French Laboratories for supplying the compounds tested.

Received for publication Oct. 27, 1954.

tested with each substance. Effective doses varied from substance to substance, but an attempt was made to standardize on 5.0 mg. as an initial amount, repeating this dose up to three times in order to observe further changes. Five compounds showed changes with doses of 1.0 to 4.0 mg. per rat; the remainder required more, the largest cumulative doses being 25 to 35 mg. in the cases of three compounds, none of which was active.

Acute toxicity of a compound or depression of the myocardium was roughly estimated by examination of the records for abnormal pulse tracings and the presence of depressor effects in both types of rats. Although both systolic and diastolic pressures were measured, only diastolic changes were here considered, being rough indices of peripheral resistance in the absence of changes of cardiac output. Results were not utilized when pulse pressures became small or pulses irregular.

In order to ascertain the variability of the method, saline solution (0.5 ml.) was injected into 85 unoperated normotensive rats with diastolic pressures of 60 to 100 mm. Hg. An insignificant change in mean blood pressure occurred in the subsequent twenty minutes. At five minutes the average rise was 0.04 mm. with a standard deviation of 13.9; At ten minutes there was a fall of 4.56 mm. (S.D. 13.7); at twenty minutes there was a fall of 1.83 mm. (S.D. 14.4). In 100 operated rats with diastolic pressures over 100 mm., the changes were similar; at five minutes a fall of 1.11 mm. (S.D. 15.9), at ten minutes a fall of 4.79 mm. (S.D. 19.7), and at twenty minutes a fall of 5.34 mm. (S.D. 14.8). As additional controls were the results of the injection of 25 substances into 93 normal rats and 24 substances into 101 hypertensive rats, for diastolic pressure was altered less than one standard deviation at the twenty-minute interval; in addition, 28 compounds in larger doses caused a mean fall of less than 15 mm. at the one- to two-hour interval in 102 normal animals, and 12 compounds showed a similar lack of effect in 61 hypertensive animals.

*Types of Response.*—Theoretically there can be five types of sustained depressor responses of the blood pressure of rats to the intravenous injection of various active compounds, according to the present method of test:

*Type A (antihypertensive):* A compound which lowers the blood pressure of normotensive rats little or not at all, but specifically lowers the blood pressure of anesthetized renal hypertensive rats, while the pressor action of norepinephrine is unaltered or little affected (Fig. 1). A compound was classed in this group if it caused a depression of average normal diastolic pressure of less than 15 mm. Hg one to two hours after injection, while elevated pressure fell 30 mm. or more (12 compounds), or if there was a difference of at least 35 mm. Hg in the final diastolic pressure of the two types of animals with the normal being lowered less than 20 mm. (one compound). Judging from prolonged experiments in which either nothing or saline solution was injected, a differential fall of diastolic pressure of 20 mm. Hg or more in one to two hours could be considered somewhat significant, and 30 mm. highly so, unless the animals were in poor condition. Compounds which showed differential actions of less degree were listed as "possibly active."

*Type B (sympatholytic):* A compound which affects blood pressure little if at all, but depresses the pressor action of norepinephrine in both types of rats. From a number of experiments, blockade of the pressor action of norepinephrine was considered somewhat significant when the diastolic rise caused by a standard minimal dose (usually 0.3 $\gamma$) was halved, and highly significant when it was reduced to one-quarter or less of the mean control values. There were three substances in this category. "Sympatholytic" as here used refers only to blockade of norepinephrine.

*Type C (antihypertensive and sympatholytic):* A compound which alters the blood pressure of normotensive rats little or not at all, lowers the blood pressure of hypertensive animals specifically, and abolishes or depresses the pressor action of norepinephrine in both types of animals. Standards similar to those previously described were used. There were three compounds showing these two actions.

*Type D (depressor):* A compound which lowers blood pressure in both types of rats and depresses the pressor action of norepinephrine. Under these circumstances, reactivity of vascular smooth muscle may have been depressed in some nonspecific or toxic manner.

No compounds of this nature were found in the present series, although certain derivatives of dibenamine and epinephrine have shown this type of reaction.

*Type E (depressor but not sympatholytic):* A compound which lowers the blood pressure of both normotensive and hypertensive rats without greater effect on the latter (30 mm. or more) but does not diminish the pressor action of norepinephrine (Fig. 2). Six substances fall into this category. Ganglionic blocking agents can also be expected to give this type of reaction.

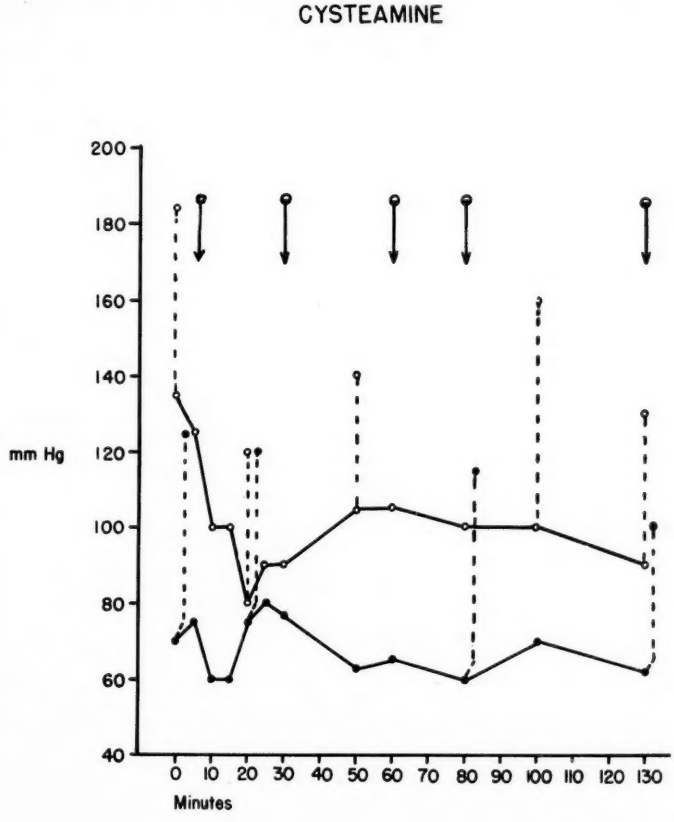



Fig. 1.—Effect of a sulfhydryl-containing substance (cysteamine) on the diastolic pressure of a normotensive and renal hypertensive rat to show typical Type A response. At the arrows 5 mg. of the substance was injected intravenously. Note the sustained fall only in the hypertensive rat. Subsequent doses produced little further change. The dotted lines indicate the elevation of diastolic pressure following the injection of 0.3 $\gamma$ of norepinephrine. No significant depression of this response occurred.

In addition, short or prolonged pressor effects were observed in normal or hypertensive animals in eight instances. However, only substances showing sustained differential actions are of interest to this study, particularly those classified as Types A, B, and C.

Certain compounds showing prolonged pharmacologic effects were given repeatedly to trained renal hypertensive dogs. Mean blood pressure was measured by a mercury manometer with direct femoral arterial puncture twenty-four hours after the material was injected. Control periods of these dogs were long. Measurements were made six days a week and charted. In a few instances the same drugs were given to human subjects. The systolic pressures of unanesthetized rats were measured by the method of Kersten and associates.[2]

The results were evaluated by comparing the mean changes in diastolic pressures of the normal and the hypertensive rats given each drug, 15 minutes and one to two hours after injection of the substance or at the end of the experiment. Data were examined for trends and discarded when it appeared that chance abnormalities had occurred. The mean rise caused by norepinephrine was also compared with the mean change after each subsequent injection.

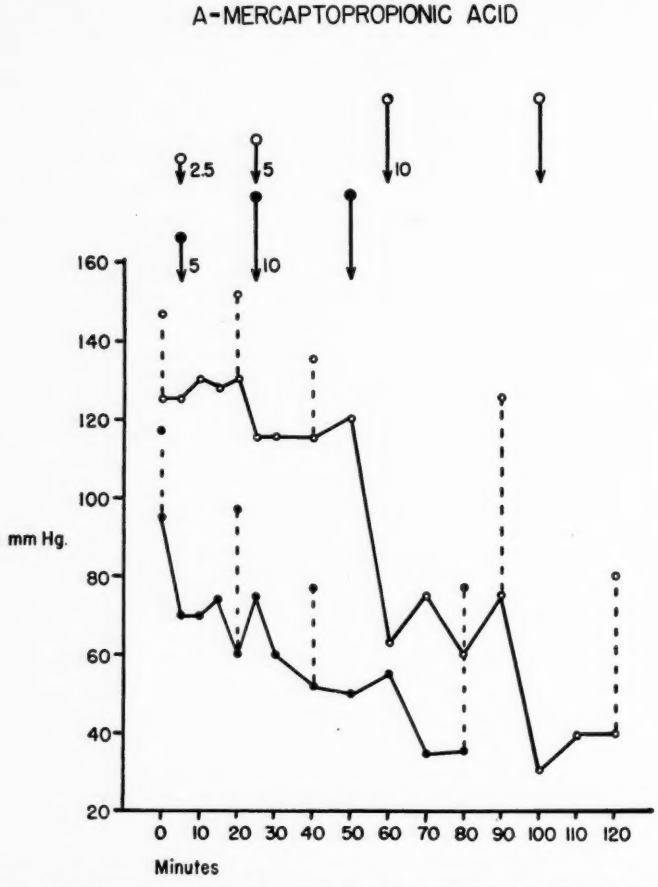



Fig. 2.—Alterations of diastolic pressure in a normotensive and hypertensive rat following the injection of α-mercaptopropionic acid to show typical Type E response. Note that the diastolic pressure of both animals fell to approximately the same level. The height of the arrows indicates the dose. The dotted lines show the elevation of diastolic pressure after 0.3 γ norepinephrine. No inhibition was observed.

## RESULTS

*Mercaptans.*—In Table I are shown the classifications of twenty aliphatic and five aromatic mercaptans as to types of responses in anesthetized rats. The ten showing differential effects upon the blood pressure of renal hypertensive as compared to normotensive rats (Types A and C) contained a free sulfhydryl group on a relatively simple structure. Apparently this effect was quite specific; for example, three salts of β-mercaptopropionic acid and four related compounds exhibited activity of this type while α-mercaptopropionic

acid was a nonspecific depressor substance. Cysteamine was active and cysteine was not; similarly, mercaptosuccinic (thiomalic) acid showed a differential effect while a complex derivative did not. Only one aromatic mercaptan was active. Two of the four listed as Type E caused considerably greater depression in hypertensive as compared to normotensive animals, one being BAL, which, given intramuscularly in oil, was depressor in both anesthetized and unanesthetized hypertensive animals, although in the latter the effect was short-lived (two to four hours) and "tolerance" appeared. Pantetheine exhibited a transient differential action. The effective doses of these compounds were large, 10 to 20 mg. per rat.

TABLE I. DIFFERENTIAL ACTION OF MERCAPTANS ON DIASTOLIC PRESSURE IN THE RAT

| COMPOUND | NO. RATS | CHANGE IN MEAN DIASTOLIC PRESSURE AFTER INJECTION (MM. HG*) | | | |
|---|---|---|---|---|---|
| | | 15 MIN. | | 60-150 MIN. | |
| | | N | O-N | N | O-N |
| *Type A Compounds (antihypertensive)* | | | | | |
| Thioglycollic acid | 10 | − 9 | −38 | −18 | −36 |
| Mercaptoethanol | 13 | + 5 | + 3 | − 4 | −26 |
| Cysteamine | 8 | − 1 | −19 | −14 | −20 |
| Procaine salt of β-mercaptopropionic acid | 6 | −21 | +31 | − 5 | −57 |
| Ethyl β-mercaptopropionate | 4 | +30 | −39 | + 5 | −39 |
| Methyl tris (β-mercaptoethyl) ammonium chloride | 8 | +36 | −21 | − 6 | −26 |
| Thiomalic acid | 7 | + 1 | −22 | + 1 | −50 |
| 1-Ethyl 2-mercaptoimidazole | 4 | − 7 | −44 | −10 | −32 |
| *Type C Compounds (antihypertensive and sympatholytic)* | | | | | |
| β-mercaptopropionic acid | 10 | − 3 | −18 | 0 | −34 |
| Glutathione, reduced | 6 | +13 | −31 | + 4 | −46 |
| *Type E Compounds (depressor but not sympatholytic)* | | | | | |
| α-Mercaptopropionic acid | 9 | − 8 | +14 | −67 | + 8 |
| Monothioglycerol | 7 | 0 | − 1 | −26 | + 3 |
| 6-amino α-mercaptobenzothiazole | 4 | −25 | +21 | −22 | −23 |
| 2,3 Dimercaptopropanol (BAL) intramuscularly in oil | 10 | +10 | + 1 | −21 | −25 |
| *Possibly active compounds* | | | | | |
| Ammonium mercaptopyruvate (C) | 7 | −17 | − 2 | −11 | −13 |
| *Inactive compounds* | | | | | |
| Cysteine | 15 | −11 | +16 | + 2 | −14 |
| 2-Mercaptoethyl hydrogen (carboxymethylmercapto) succinate | 8 | 0 | +23 | − 8 | +18 |
| Mercaptosalicylic acid | 8 | − 2 | + 2 | − 8 | + 7 |
| 1-Methyl 2-mercaptoimidazole | 10 | − 3 | + 4 | + 9 | + 9 |
| Pantetheine | 6 | −11 | −34 | − 5 | + 1 |
| 2,3 Dimercaptopropanol (BAL) intravenously in saline solution | 8 | +17 | −12 | −13 | −12 |
| Tridecyl mercaptan | 6 | +16 | + 2 | + 3 | +11 |
| Acetonyl mercaptan (B) | 4 | 0 | + 5 | + 5 | − 1 |
| Thiohydroxypropane (B) | 4 | − 1 | − 4 | − 7 | +12 |
| Sodium 2-mercapto-5-benzothiazole sulfonate (B) | 15 | − 9 | + 6 | − 7 | +10 |

*The mean depression of diastolic pressure 15 and 60 to 150 minutes after injection of a compound is shown for normal rats (N) as well as the differences between normal and renal hypertensive rats (O-N). Mean depression of diastolic pressure in hypertensive animals can be obtained by adding the two figures. The change at 15 minutes represents the effects of one 5.0 mg. dose, at 60 to 150 minutes that of two or three subsequent 5.0 mg. doses. Systolic pressures altered comparably. See text for definitions of types of responses.

One compound of interest contained three ethyl mercaptans on a quaternary ammonium nitrogen; it was made on the possibility that ganglionic blockade as well as mercaptan effect might result. However, it acutely raised the mean diastolic pressures of normal and hypertensive rats 36 and 15 mm. Hg, respectively, producing the usual differential mercaptan effect of a depression of 6 and 32 mm., respectively, at the end of two hours. Significant pressor effects were seen with the succinate derivative, tridecyl mercaptan, and in normal animals with the ethyl ester of β-mercaptopropionic acid.

TABLE II. ACTION OF COMPLEXES CONTAINING OXIDIZED SULFUR ON DIASTOLIC PRESSURE

| COMPOUND | NO. RATS | CHANGE IN MEAN DIASTOLIC PRESSURE AFTER INJECTION (MM. HG*) | | | |
|---|---|---|---|---|---|
| | | 15 MIN. | | 60-150 MIN. | |
| | | N | O-N | N | O-N |
| *Type A Compounds* | | | | | |
| Thiosemicarbazide | 7 | + 4 | –19 | – 7 | –35 |
| Tetrathiodiacetic acid | 3 | +23 | –23 | +23 | –65 |
| 2-t-Butyl-2-thiopseudourea HCl | 5 | +18 | –10 | + 3 | –45 |
| Ethyl (guanylmercapto) acetate HCl | 4 | –25 | +16 | 0 | –35 |
| Pseudothiohydantoin | 10 | – 8 | –12 | –12 | –46 |
| *Type C Compounds* | | | | | |
| Trithio-paramethoxyphenyl propene (SKF-1717) | 5 | +23 | –63 | +25 | –55 |
| *Type E Compounds* | | | | | |
| 2-Pentyl-2-thiopseudourea propionate | 5 | –22 | +25 | –38 | +17 |
| 2-Benzyl-2-thiopseudourea HCl | 4 | –13 | + 5 | –45 | –20 |
| Hexamethylene-bis [2-(guanylmercapto) ethyl] dimethyl ammonium chloride dihydrochloride | 9 | –24 | –48 | –36 | –50 |
| *Inactive Compounds* | | | | | |
| Cystine | 12 | – 5 | – 2 | – 7 | – 2 |
| Methionine | 13 | – 5 | + 6 | 0 | + 3 |
| Glutathione, oxidized | 13 | 0 | – 3 | + 2 | + 1 |

*The mean depression of diastolic pressure 15 and 60 to 150 minutes after injection of a compound is shown for normal rats (N) as well as the differences between normal and renal hypertensive rats (O-N). Mean depression of diastolic pressure in hypertensive animals can be obtained by adding the two figures. The change at 15 minutes represents the effects of one 5.0 mg. dose, at 60 to 150 minutes that of two or three subsequent 5.0 mg. doses. Systolic pressures altered comparably. See text for definitions of types of responses.

*Compounds of Oxidized Sulfur.*—In Table II are shown the types of responses produced by 11 compounds containing sulfur, most of which also contained nitrogen in their molecules. An examination of their structural formulas revealed that all but two of Types A and C contained sulfur, carbon, and nitrogen adjacent to an aliphatic chain or in a ring with the nitrogen in a double bond (S—C=NH); the exceptions were tetrathiodiacetic acid and the trithione with sulfur in a double bond. None of the inactive compounds contained this configuration. Prolonged depressor effects, whether differential or not, were exhibited by all compounds with these structures except one, which apparently blocked norepinephrine. Effective doses were usually large, 5 to 20 mg., although changes occurred with the trithione in 2.0 mg. amounts and with the benzyl thiopseudourea in 5 mg. amounts.

One of special interest had the basic structure of hexamethonium ion with an ethyl thiopseudourea group on each quaternary nitrogen. Although listed

as Type E, it depressed the mean diastolic pressure of five normal rats 36 mm. Hg and that of five hypertensive rats 86 mm. in doses of 1.0 to 1.5 mg. Possibly ganglionic blockade was combined with another action.

In order to test for effect as related to structure, six new compounds synthetized by Dr. Earle Scott were given to pairs of rats in twenty experiments. All showed some activity. Type A effects were exhibited by two, $H_2NCSNHNHCSN_2H_3$ and its benzyl derivative, $\phi NHNHCSN_2H_3$, while alteration of the terminal nitrogen to amide changed the effect to Type E. Three five-membered ring structures with S—C=N in their molecules or sulfur in a double bond were differentially active and partially blocked norepinephrine.

*Sympatholytic Effects.*—In Table III are shown the eight compounds exhibiting sympatholytic activity as manifested by depression of the pressor action of norepinephrine. None of the other thirty-four compounds tested in a similar manner exhibited this type of activity, whether depressor or not. The doses necessary were large, 10 to 20 mg. per rat.

TABLE III. AGENTS BLOCKING NOREPINEPHRINE

| COMPOUND | MEAN DEPRESSION OF PRESSOR EFFECT OF NOREPINEPHRINE (%) |
|---|---|
| *Type B (not depressor)* | |
| Acetonyl mercaptan | 100* |
| Thiohydroxypropane | 80* |
| Sodium 2-mercapto-5-benzothiazole sulfonate | 85* |
| *Type C (antihypertensive and "sympatholytic")* | |
| β-Mercaptopropionic acid | 80 |
| Glutathione (reduced) | 100* |
| Trithione (SKF-1717) | 91 |
| *Possibly sympatholytic* | |
| Ammonium mercaptopyruvate (C) | 56 |
| Tridecyl mercaptan | 56 |

*Reversal of pressor action observed in some experiments.

*Experiments in Renal Hypertensive Dogs.*—A few of the substances were given to renal hypertensive dogs. Thiosemicarbazide orally was toxic, causing fits and death. Cysteine (100 mg. intravenously) daily for ten days was without demonstrable effect. Pseudothiohydantoin (450 mg. intravenously) daily for ten days in one dog resulted in an insignificant (10 mm.) fall in average mean pressure. BAL (5 mg. per kilogram) injected intramuscularly produced definite but transient (two- to six-hour) depression of blood pressure, as did sodium thioglycollate intravenously. When BAL was injected with 1-hydrazinophthalazine, a moderately active depressor substance, the effects were enhanced (Fig. 3).

*Experiments in Hypertensive Patients.*—Only four of these compounds were studied sufficiently to give to human beings. Cysteine, in doses up to 1.0 Gm. intravenously, caused no demonstrable or significant alteration in the blood pressure of four hypertensive patients, even when given daily for one week. Monothioglycerol in doses of 300 mg. to 1.0 Gm. intravenously in three seriously ill hypertensive patients was apparently inactive in this respect,

diastolic pressure falling only 10 to 15 mm. Hg. A trithione (SKF-1717) given orally to five hypertensive patients in large doses appeared inert. BAL, however, exhibited depressor activity in six hypertensive patients when given every four hours in doses up to 5.0 mg. per kilogram; the effects were relatively short lived (two to four hours). We did not observe a pressor effect after this material was given.

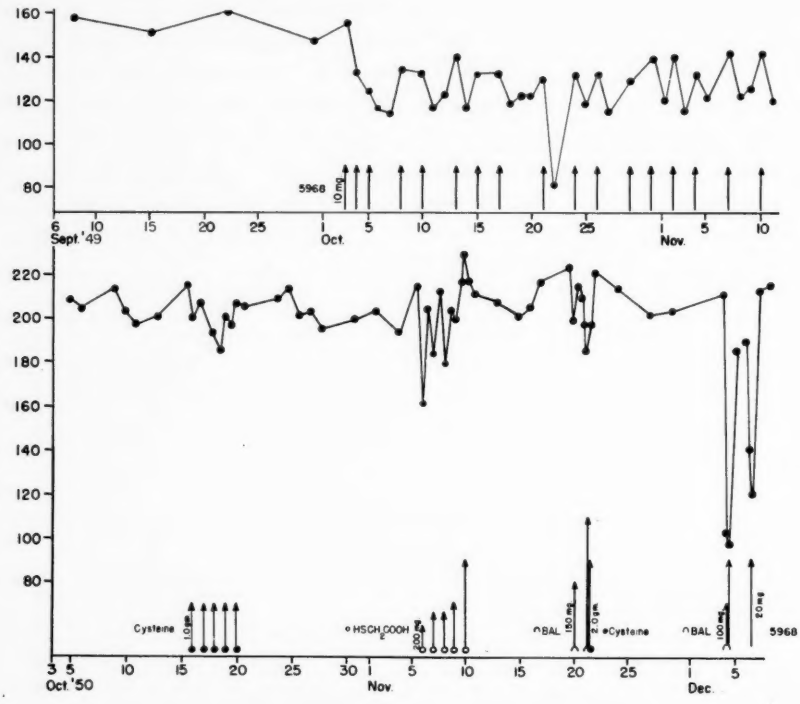



Fig. 3.—Effect of various substances on the mean blood pressure of a renal hypertensive dog. The left renal artery was constricted in August, 1949, causing moderate hypertension. Upper: 1-Hydrazinophthalazine (5968) injected intravenously. Note the effect when given at less frequent intervals than daily, blood pressure falling for 24 hours and then rising in 48. Only small doses were required. During the subsequent year mean blood pressure rose. Lower: Cysteine intravenously caused only insignificant variations; sodium thioglycollate caused more pronounced but transient ones; BAL intramuscularly alone and combined with intravenous cysteine caused similar depressions; but BAL combined with 5968 produced marked responses. Both the immediate (2- to 6-hour) and late (24-hour) responses to these substances are shown. Only BAL and 5968 together produced significant depression for 24 hours. The dog's mean blood pressure consistently remained above 200 mm. Hg for five months after this study, when it was given 1.0 Gm. thiosemicarbazide by mouth and died of convulsions.

## DISCUSSION

While the anesthetized rat makes a preparation for pharmacologic testing which is somewhat unstable, it has the advantage of requiring small amounts of material and of readily being made hypertensive. Experience with this animal has indicated that, properly evaluated, information of a limited variety may be obtained in brief experiments. Those here reported were controlled by the injection into rats of a very large number of chemical substances other than those listed. Although the level of blood pressure is a function of both cardiac output and peripheral resistance and although cardiac effects cannot

be excluded by simple measurements of mean blood pressure, inspection of records rapidly recorded by a suitable manometer will usually reveal changes in pulse rate or in the shape, size, or regularity of the pulse curves, suggesting alterations in factors other than peripheral resistance. The method is, therefore, valid within limits and with proper interpretation.

Two possibilities exist for the differential effects of some of these compounds upon the blood pressure of anesthetized renal hypertensive rats. First, their vascular systems may be more reactive than those of normal animals to subeffective doses of nonspecific depressor substances. While this possibility cannot be ruled out, attempts were made to control it in a number of instances by giving larger doses to normal rats, without observed effect. The variety of compounds tested, as well as the various types of responses produced, also serve as controls. Our total experience includes over 100 compounds screened in this manner.

The second is that some direct or indirect action upon a fundamental mechanism altered in renal hypertensive rats was affected. The apparent consistency of the responses to aliphatic mercaptans and to thiopseudoureas suggests that a chemical reaction took place which lowered elevated blood pressure but did not alter normal blood pressure. Analogous responses have been reported for sodium azide[3] and BAL[4] (shown to be pressor in normal human beings[5]).

Examination of the formulas of the compounds here considered gave some clue as to their possible mode of action. Most of them with a free sulfhydryl group on an aliphatic carbon chain were depressor on hypertensive or on both types of rats. In addition, compounds which might be metabolized to sulfhydryl or which contained S—C=NH in a chain or ring exhibited activity; either free mercaptan or sulfur near a double bond appeared essential.

Blockade of the pressor action of norepinephrine occurred with both types of compounds. We are at a loss to explain this finding. It is interesting, however, that none of these substances exhibited Type D activity (depressor in both types of animals plus blockade), a quality observed with certain depressor substances. $\beta$-Mercaptopropionate also blocks other primary amines as well as angiotonin.[6]

BAL was curious in that watery solutions given intravenously exhibited insignificant effects in rats, while oily solutions given intramuscularly lowered the blood pressure in anesthetized and unanesthetized hypertensive rats, dogs, and patients. We cannot account for this phenomenon. Our results diverge from those reported by Baylis and Kauntze[7] in human beings and agree with the experience of Modell, Gold, and Cattell[4]; perhaps dosage was the deciding factor. It should be pointed out, however, that the effects usually lasted only a few hours and seldom more than four.

These results indicate that it is possible that certain compounds of sulfur in a linkage available for metabolic processes may exhibit antihypertensive properties in rats and dogs. The mechanism of action is obscure. Some of

these substances may bind trace metals, supply reducing groups, or react with enzymes. Their toxicities and actions in chronic experiments have not been determined.

## SUMMARY AND CONCLUSIONS

Thirty-six compounds containing sulfur in their molecules were screened for prolonged depressor properties in anesthetized normal and renal hypertensive rats; a few were also tested in dogs and human subjects. Sixteen showed differential depressor effects only on hypertensive rats, six apparently blocked the pressor action of norepinephrine, seven were nonspecific depressor substances, two showed questionable activity, and nine were inactive. Those exhibiting "specific" activity in hypertensive animals or blocking norepinephrine contained either free sulfhydryl groups, thiopseudourea, sulfur in a double bond, or S—C=NH in a ring. It is possible that certain of these compounds affect some mechanism altered in renal hypertensive rats.

## REFERENCES

1. Schroeder, H. A., and Olsen, N. S.: Pressor Subtances in Arterial Hypertension. II. Demonstration of Pherentasin, a Vasoactive Material in Blood, J. Exper. Med. **92**: 545, 1950.
2. Kersten, H., Brosene, W., Ablondi, F., and Subbarow, Y.: A New Method for the Indirect Measurement of Blood Pressure in the Rat, J. LAB. & CLIN. MED. **32**: 1090, 1947.
3. Black, M. M., Zweifach, B. W., and Speer, F. D.: Comparison of Hypotensive Action of Sodium Azide in Normotensive and Hypertensive Patients, Proc. Soc. Exper. Biol. & Med. **85**: 11, 1954.
4. Modell, W., Gold, H., and Cattell, McK.: Clinical Uses of 2,3-Dimercaptopropanol (BAL). IV. Pharmacologic Observations of BAL by Intramuscular Injection in Man, J. Clin. Invest. **25**: 48, 1946.
5. Sulzberger, M. B., Baer, R. L., and Kanof, A.: Clinical Uses of 2,3-Dimercaptopropanol (BAL). III. Studies on the Toxicity of BAL on Percutaneous and parenteral Administration, J. Clin. Invest. **25**: 474, 1946.
6. Schroeder, H. A.: The Antihypertensive Influence of Certain Sulfhydryl Compounds, Science **114**: 441, 1951.
7. Baylis, J. H., and Kauntze, R.: The Effect of Dimercaprol on Hypertension, Lancet **1**: 1092, 1952.

# FURTHER STUDIES OF A HEMOSTATIC DEFECT CAUSED BY INTRAVENOUS DEXTRAN

EDWARD ADELSON, CAPTAIN, MC, WILLIAM H. CROSBY, LIEUTENANT COLONEL, MC, AND WILLIAM H. ROEDER, CORPORAL, AMEDS
WASHINGTON, D. C.

WITH THE TECHNICAL ASSISTANCE OF CAROL E. THIBEAULT

DEXTRAN, a high molecular weight polymer of dextrose, was developed as a plasma substitute. Recent studies carried out at the Army Medical Service Graduate School have shown a tendency toward prolonged bleeding time in every subject receiving large amounts of commercially available dextran[1] and in 28 per cent of subjects receiving 1,000 ml. of dextran.[2]

Examination of various factors in the coagulation mechanism of animals and human beings who have received dextran reveals some changes which will be reported elsewhere. None of these changes are of sufficient severity to explain the prolonged bleeding time. However, a positive tourniquet test has been noted at the height of the bleeding tendency.[3] Since tourniquet test and bleeding time are both dependent on the integrity of the platelet, the vessel wall, or both, we believe that the basic postdextran defect must be in these areas.

Because platelets are directly involved in hemostasis, thrombocytopenia from any cause would be expected to aggravate a tendency to bleed. It seemed desirable to learn whether radiation-induced thrombocytopenia would increase the tendency to abnormal bleeding that follows the use of dextran. The following study was planned to answer that question.

## METHODS

Six dogs, all males of approximately equal weight (12 to 14 kilograms), were used in this study. Determination of bleeding time in dogs was carried out with a No. 11 Bard-Parker blade anchored in a cork stopper, the point of the blade extending exactly 5 mm. beyond the stopper. The cut was made on the shaved surface of the anterior abdomen in the following manner: A fold of skin and subcutaneous tissue was grasped between thumb and index finger and lifted up from the underlying abdominal muscle. A stab wound was made through the fold of skin perpendicular to the underlying muscle to the full 5 mm. depth of the blade. In this way a standard reproducible cut could be made. Normal dogs do not bleed longer than one or two minutes after such a cut, and often they do not bleed at all.

Platelet counts were carried out by the method of Brecker and Cronkite.[4] The normal platelet count in dogs ranges from 300,000 to 400,000 and in human beings, from 200,000 to 300,000.

In human beings bleeding time was carried out by the Duke method. In this method, a glass slide is placed behind the ear lobe. Then a stab wound is made through the ear lobe with a No. 11 Bard-Parker blade. A definite click must be heard as the blade completely pierces the lobe and strikes the glass slide. In this method a 3 to 5 minute bleeding time is normal.

From the Department of Hematology, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.
Received for publication Oct. 25, 1954.

Dextran plasma levels were determined by the method of Metcalf and Rousselot.[6] All dextran referred to was R. K. Laros Company Dextran, Lot 13301A. This particular lot was the one which most often caused bleeding tendency in a previous study on human beings.[2] A course of dextran for the dogs consisted of 250 ml. of a 6 per cent solution per day for 3 days or until bleeding time prolongation was produced. At least 3 weeks (and usually longer) elapsed between successive courses.

X-ray exposure* was accomplished via a 2 mev. roentgen tube. The dogs received 62.5 r. per side for a total dose of 125 r. The animals had no significant drop in red count, hematocrit, or white count. About 10 days after irradiation the platelet count reached a plateau of 100,000 to 150,000 and remained at this plateau during the subsequent 3 weeks. This pattern of response is consistent with previous reports by Cronkite and Brecker.[5] During the plateau period, the postirradiation studies were carried out.

The six dogs were divided into 3 groups of 2 each, as shown in Table I. Group 1 received repeated courses of dextran. Then a total body irradiation dose of 125 r. was administered. When the animals reached the thrombocytopenic plateau previously described, the response to dextran was restudied.

TABLE I. TREATMENT GIVEN TO EACH OF THE THREE GROUPS OF DOGS—TWO DOGS IN EACH GROUP

| GROUP | DEXTRAN PRIOR TO IRRADIATION | X-RAY | DEXTRAN AFTER IRRADIATION | GELATIN AFTER IRRADIATION |
|---|---|---|---|---|
| 1 | + | + | + | 0 |
| 2 | + | + | 0 | + |
| 3 | + | 0 | + | 0 |

Group 2 was treated the same as Group 1, except that after x-ray the animals received gelatin instead of dextran. A course of gelatin consisted of a daily infusion for 3 successive days of 250 ml. of 3 per cent modified gelatin in 0.7 per cent NaCl, Lot MFG-9, as prepared by Charles B. Knox Company.

Group 3 was treated the same as Group 1, except that no irradiation was administered.

## RESULTS

The thrombocytopenic animals clearly showed increased sensitivity to the effect of dextran on bleeding time. Figs. 1 and 2 show the response of Dogs 1 and 2, respectively, in Group A. Figs. 3 and 4 show the response of Dogs 3 and 4, respectively, in Group B. Fig. 5 shows the response of Dogs 5 and 6 in Group C. Fig. 6 shows the response of dogs in Group A and B to an additional course of dextran therapy after they had completely recovered from all effects of the irradiation.

Prior to irradiation, when the platelet counts averaged 300,000 to 400,000 per cu. mm., the two dogs in Group A showed the usual sensitivity to dextran; that is, an average of 15 Gm. (250 ml. of 6 per cent) dextran per day for three days was required before they developed prolongation of bleeding time.

Ten days postirradiation, when the platelet counts had dropped to an average of 150,000 per cu. mm., both dogs developed marked prolongation of bleeding time after single infusions of 15 Gm. of dextran. Of course, dilution by this single infusion further lowered the platelet count from an average preinfusion level of 150,000 to an average postinfusion level of 55,000. It might be argued from this that thrombocytopenia alone could explain the

*We are indebted to Commander Eugene R. Cronkite of the Naval Medical Research Center, Bethesda, Md., for carrying out this irradiation.

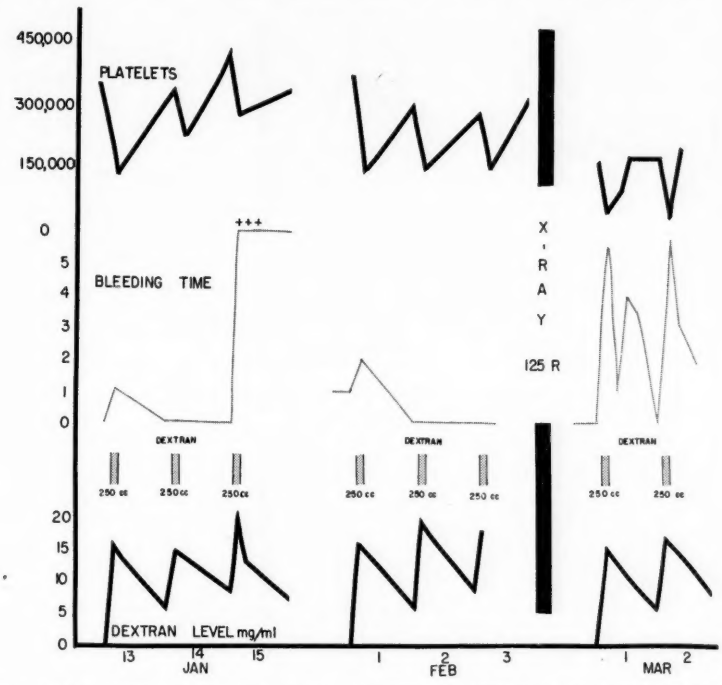



Fig. 1.—This chart shows that Dog 1 (Group A) developed prolonged bleeding only after 750 ml. of dextran on his first trial, and did not develop prolonged bleeding after 750 ml. on his second trial. After irradiation he developed prolongation of bleeding time after only 250 ml. of dextran.

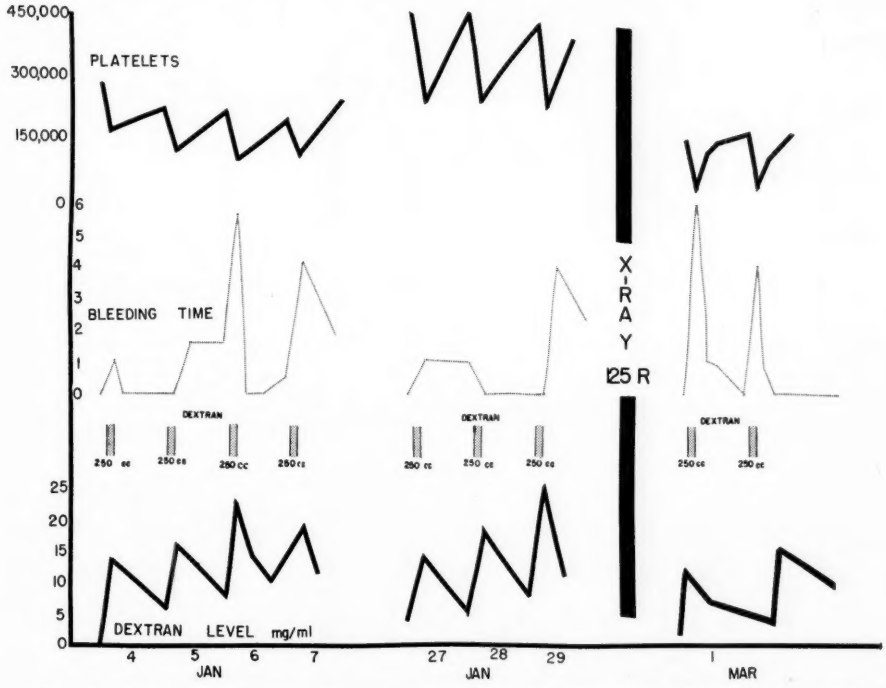



Fig. 2.—This chart shows that Dog 2 (Group A) on two trials required 750 ml. of dextran to produce prolonged bleeding time before irradiation. After irradiation 250 ml. produced prolonged bleeding time.

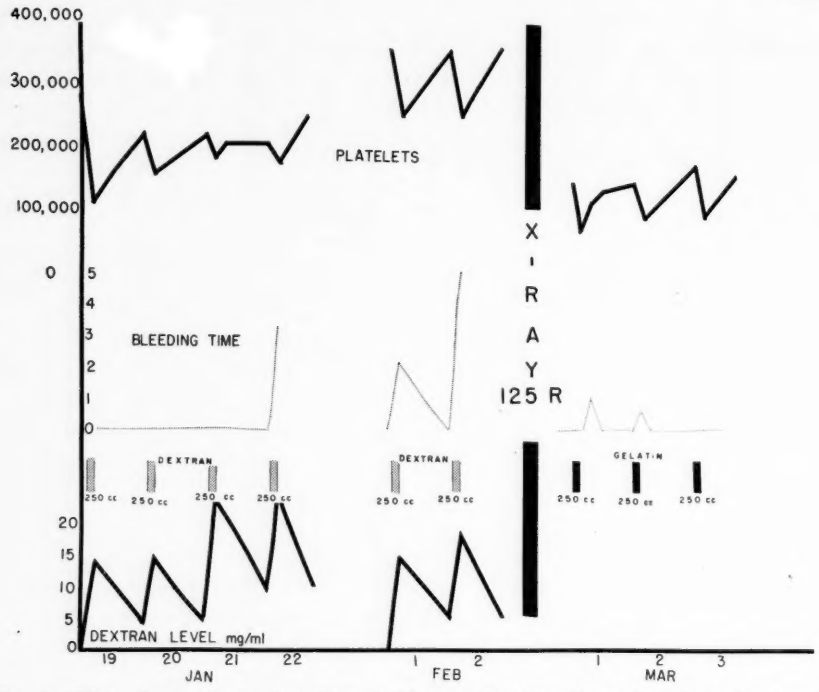



Fig. 3.—This chart shows that Dog 3 (Group B), like Dogs 1 and 2, developed post-irradiation thrombocytopenia. The infusions of gelatin further diluted the platelet counts—just as the postirradiation dextran had diluted the platelet counts in Dogs 1 and 2. Yet, no bleeding time prolongation occurred. This demonstrates that the increased sensitivity in Dogs 1 and 2 after irradiation was not due to thrombocytopenia alone, but to thrombocytopenia plus dextran.

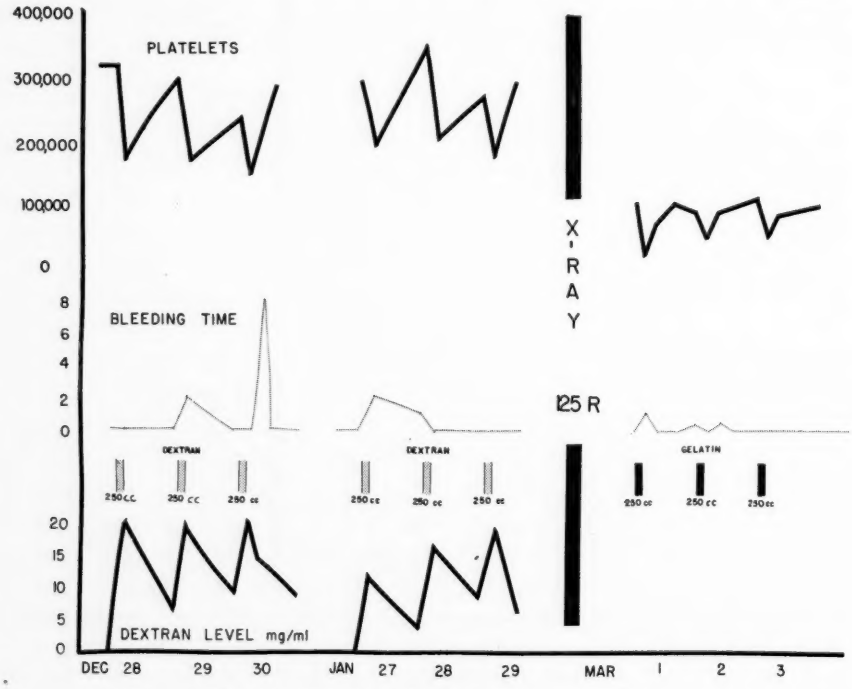



Fig. 4.—Dog 4 (Group B). Results similar to Dog 3 (Group B).

hemostatic defect. However, the two dogs in Group B rule out this possibility. These two dogs also showed average sensitivity to dextran prior to irradiation. After irradiation, their platelet counts were 140,000 (Dog. 3) and 110,000 (Dog 4). They received I.V. gelatin and their platelet levels dropped (via dilution) to 82,000 and 48,000, respectively. Yet, they showed no hemostatic defect after these infusions.

The relatively constant pattern of response to dextran shown by the dogs in Group C proves that passage of time or increasing sensitivity secondary to repeated dextran infusions cannot explain the results in Group A.

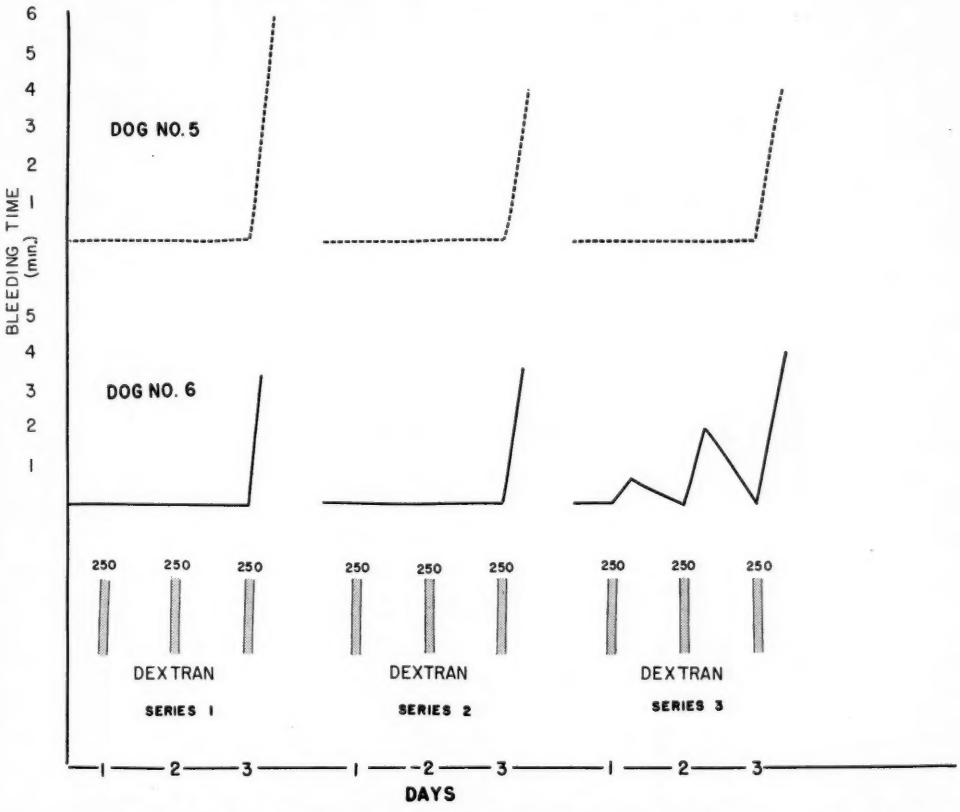



Fig. 5.—Dogs 5 and 6 (Group C). These results show that repeated courses of dextran infusion do not lead to increasing sensitivity to dextran. This is a further control for the results in Group A.

## DISCUSSION

Prolongation of bleeding time and positive tourniquet test develop in many patients who have received dextran therapy. This is evidence of a platelet or vessel wall defect.

The one patient who proved most sensitive to the prolongation of bleeding time after dextran was a man who had an unexplained moderate thrombocytopenia. This patient's platelet count averaged 110,000 by the Brecker and Cronkite method. Prior to dextran his bleeding time was normal by the Duke

method. His response to dextran, as compared to the response of the usual individual susceptible to the prolonged bleeding time, is shown in Fig. 7. The extreme duration of hemostatic defect in this patient was not due simply to further drop in platelet count secondary to the dilution effect of the plasma

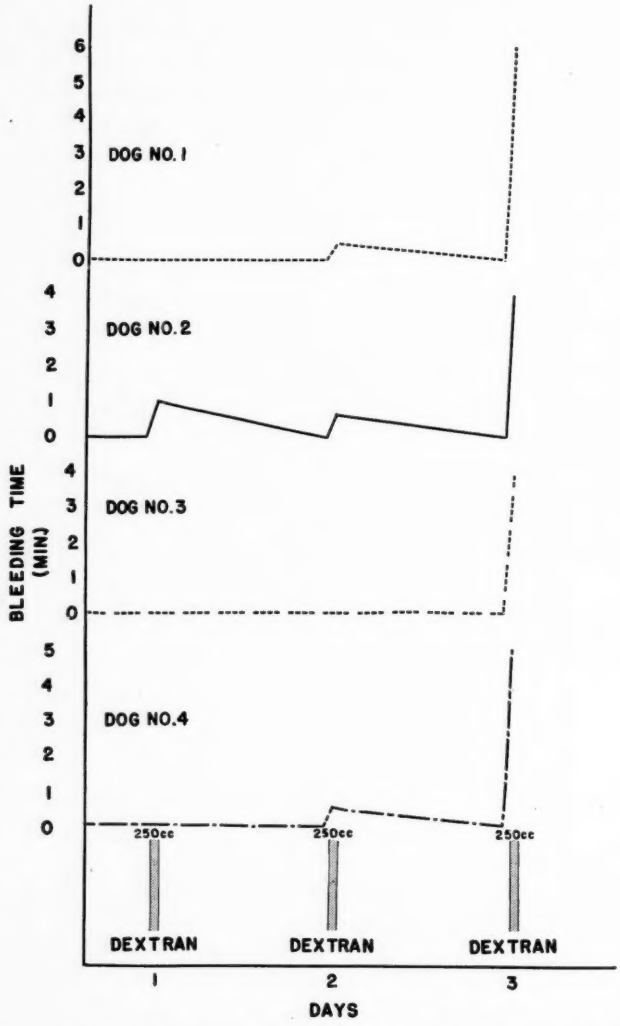



Fig. 6.—Groups A and B, Dogs 1, 2, 3, and 4. This chart shows the response of these dogs to a course of dextran given after the postirradiation thrombocytopenia had disappeared. The results indicate that sensitivity returns to normal after the thrombocytopenia subsides.

expander. This is shown in Fig. 8. In this chart we see that the dilution effect of dextran on the plasma, as revealed by the fibrinogen level, lasted only a few hours, whereas the bleeding time prolongation lasted four days. Furthermore, I.V. albumin produced a similar dilution effect without causing bleeding time prolongation.

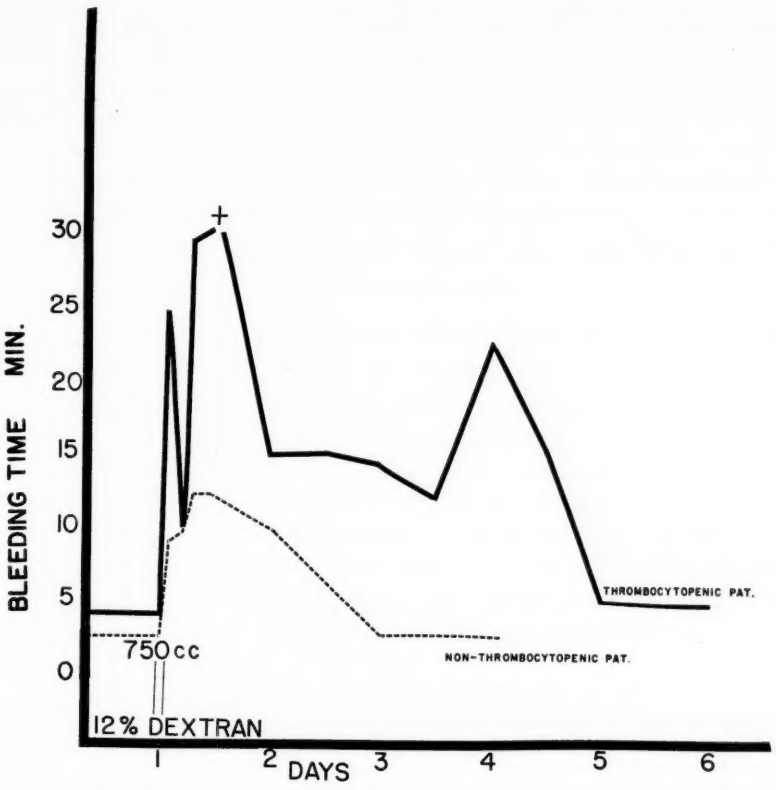



Fig. 7.—Response of a moderately thrombocytopenic patient compared with the response of a nonthrombocytopenic patient to equal volumes of I.V. dextran. The thrombocytopenic patient shows a much more marked response—both in severity and duration.

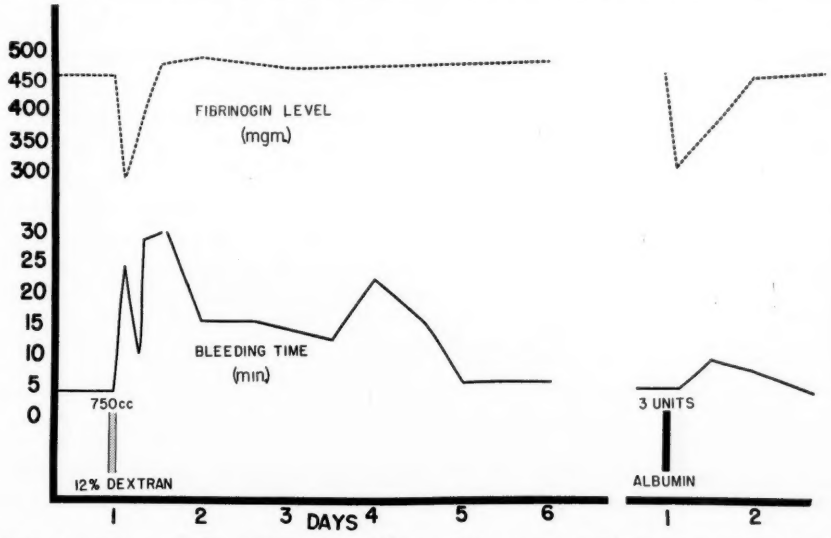



Fig. 8.—The markedly prolonged duration of the hemostatic defect produced by dextran in a moderately thrombocytopenic patient. This chart shows that the defect long outlasts the transient hemodilution which is indicated by the dip in the fibrinogen level. Furthermore, a comparable dilution produced by I. V. albumin did not result in a prolongation of bleeding time.

The dog irradiation experiment furnishes further evidence of the importance of platelet level on the effect of dextran on bleeding time. Dogs 1 and 2 both showed increased sensitivity to dextran after they had become moderately thrombocytopenic. Dogs 3 and 4 showed that this bleeding time prolongation is not due to the thrombocytopenia alone or to the dilution effect of the plasma expander alone. Dogs 5 and 6 showed that this bleeding time prolongation is not due to increased sensitivity to dextran after repeated courses. In fact, all 6 groups showed remarkable constancy in their response to dextran, except after the thrombocytopenia of irradiation.

## SUMMARY AND CONCLUSIONS

Previous reports[1, 2] have pointed out the occurrence of a prolonged bleeding time after dextran infusions. We feel that the defect is either in the platelet or vessel wall because: (a) Bleeding time depends mainly on the adequacy of platelets and vessel walls. (b) The patients develop a positive tourniquet test as part of their hemostatic defect. (c) The patient most sensitive to the action of dextran on bleeding time had moderate thrombocytopenia. Simple dilution did not seem to explain the effect of dextran. (d) Dogs are more sensitive to dextran after moderate postirradiation thrombocytopenia. Here, too, simple dilution does not seem to explain the effect.

## REFERENCES

1. Carbone, John V., Furth, Frank W., Scott, Russell, Jr., and Crosby, William H.: An Hemostatic Defect Associated With Dextran Infusion, Proc. Soc. Exper. Biol. & Med. **85**: 101, 1954.
2. Furth, Frank W., Carbone, John V., Fox, Arthur S., and Jablon, Seymour: A Comparative Study of the Effect of Dextran PVP, and Serum Albumin Infusions on Bleeding Time in 121 Normal Subjects. To be published.
3. Adelson, Edward, Crosby, William H., Roeder, W., and Carbone, John V.: Further Studies on the Effect of Dextran on Bleeding Time, Report to National Research Council Subcommittee on Blood and Related Problems.
4. Brecker, George, and Cronkite, Eugene P.: Morphology and Enumeration of Human Blood Platelets, J. Appl. Physiol. **3**: 365, 1950.
5. Cronkite, Eugene B., and Brecker, George: Fifth Conference on Blood Clotting and Allied Problems, New York, 1952, Josiah Macy, Jr., Foundation, p. 171.
6. Metcalf, William, and Rousselot, Louis M.: A Simple, Accurate, and Rapid Method for the Quantitative Determination of Dextran in Blood and Urine, J. LAB. & CLIN. MED. **40**: 901, 1952.

# DELAYED HYPERSENSITIVITY IN EXPERIMENTAL FUNGUS INFECTIONS

## The Skin Reactivity of Antigens From the Yeast Phase of Histoplasma Capsulatum

James E. Dyson, Jr., M.S., and E. Edward Evans, Ph.D.
Ann Arbor, Mich.

HISTOPLASMIN, a filtrate from mycelial cultures of *Histoplasma capsulatum,* is commonly used as a skin test antigen in epidemiologic surveys and as an aid in the differential diagnosis of mycotic diseases. Many investigators have reported cross-reactions occurring between *H. capsulatum* and other pathogenic fungi,[1-4] and it would be desirable to eliminate such nonspecific reactions where possible in order to improve the accuracy of the skin test. Howell[2] suggested dilution of the antigen as a means for the elimination of cross reactivity. This method is not entirely satisfactory because of individual differences in host reactivity. In order to secure specificity, the histoplasmin must be diluted to the point where it loses sensitivity and some infected individuals fail to react. Since the cross-reactivity of fungal antigens has been well established, it would seem desirable to search for antigens that are specific for *H. capsulatum* in the skin test. Previous work on the fractionation of antigens from *H. capsulatum* has been done with histoplasmin from mycelial phase cultures.[5-8] This work has been summarized in a preliminary report.[9]

When the present investigation was initiated, the hypothesis was adopted that it would be more logical to search for an antigen of high sensitivity and high specificity in the yeast phase since this is the form seen in infected tissues. The fractionation of yeast cells and of cultural supernatant fluid and the assay of skin test reactivity of these antigens are described in this report.

### MATERIALS AND METHODS

The four strains of *H. capsulatum* used in these studies were designated H1, H2, H9, and H12.* All except H2 were isolated from human patients; H2 was isolated from a dog. Cultures were maintained in the yeast phase by use of a neopeptone dialysate medium.[9] This medium was dispensed in 2 L. Erlenmeyer flasks which were incubated at 37° C. on a rotary action flask shaker at 180 r.p.m. Cultures showing evidence of contamination or less than 95 per cent yeast phase cells were discarded.

---

From the Department of Bacteriology, University of Michigan Medical School, Ann Arbor, Mich.

This investigation was supported by a research grant (E436) from the National Institutes of Health, Public Health Service.

The major portion of the work described in this article has been included in a thesis presented by the senior author to the Horace H. Rackham School of Graduate Studies in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Received for publication Nov. 1, 1954.

*The cultures were received from the following laboratories:
H1 — Department of Dermatology, University of Michigan Medical School.
H2 — Dr. S. B. Salvin, Rocky Mountain Laboratory, National Institutes of Health.
H9 — Miss Charlotte C. Campbell, Army Medical Service Graduate School.
H12 — Dr. C. W. Emmons, National Institutes of Health.

Animals were infected with $5 \times 10^6$ yeast phase cells per kilogram of body weight. Rabbits were injected intravenously and guinea pigs intraperitoneally. All animals were demonstrated to be negative to histoplasmin and blastomycin before infection. Other groups of animals were infected with similar doses of yeast cells of *Blastomyces dermatitidis, Cryptococcus neoformans, Sporotrichum schenckii,* and *Candida albicans,* as well as with spore suspensions of *Coccidioides immitis.*

Skin tests were conducted at weekly intervals by the injection of 0.1 ml. of saline solution containing the desired concentration of antigen. Observations were made at short intervals after injection to detect possible immediate reactions and at 24 and 48 hours. Animals reported as positive in the tables are those in which a delayed reaction occurred with a zone of induration exceeding 3 mm.

Blastomycin and histoplasmin prepared from mycelial cultures were kindly furnished by Parke, Davis and Company, and Eli Lilly and Company. Histoplasmin was also furnished by the National Institutes of Health.

Yeast phase antigens were isolated from two sources, the cells and the cultural supernate. Details of the isolation of somatic antigens are summarized in Table I and will be omitted from the test since none of these antigens fulfilled the criteria of simultaneous high sensitivity and high specificity.

To prepare antigens by ethanol precipitation, six-day cultures were killed with 1 per cent phenol or 1 per cent formalin. In addition, some preparations were made from live cultures. The cells were removed by filtration through a Seitz sterilizing pad and the clear supernate was reduced to approximately 10 per cent of the original volume by per-evaporation at room temperature. A precipitate was secured by the addition of 10 per cent sodium acetate, 1 per cent acetic acid, and 50 per cent ethanol. After removal of this precipitate by centrifugation, additional precipitates were secured by raising the concentration of ethanol to the percentages shown in Table II. The precipitates were dissolved in water and reprecipitated using the initial ethanol concentration. All precipitations were conducted at 5° C.

All samples were dialyzed against distilled water at 5° C. until free from inorganic ion and dried by lyophilization. Fractions were numbered in chronological order of preparation. Protein was removed from Fraction 135 by shaking an aqueous solution with chloroform.[10]

TABLE I. REACTIVITY OF SOMATIC ANTIGENS FROM YEAST PHASE *H. Capsulatum*

| ANTIGEN NUMBER | ISOLATION PROCEDURE | CONCENTRATION (μG)† | SKIN REACTIONS IN RABBITS | | | |
|---|---|---|---|---|---|---|
| | | | *B. dermatitidis*-INFECTED | | *H. capsulatum*-INFECTED | |
| | | | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) |
| 211 | G* | 100 | 3/3 | 13 | 5/5 | 14 |
| 112 | G, $E_{50}$ | 10 | 0/11 | – | 0/9 | – |
| 118 | G, $E_{67}$ | 10 | 0/11 | – | 3/9 | 6 |
| 122 | G, $E_{75}$ | 10 | 0/11 | – | 1/9 | 6 |
| 217 | G, $S_{30}$ | 10 | 7/11 | 6 | 5/9 | 7 |
| 218 | G, $S_{60}$ | 10 | 10/11 | 8 | 8/9 | 5 |
| 219 | G, Z | 10 | 0/11 | – | 2/9 | 6 |
| | | 100 | 3/11 | 8 | 9/9 | 16 |
| Reference | | 10 | 0/11 | – | 9/9 | 8 |
| Fraction 200 | | 1 | 0/1 | – | 6/9 | 4 |
| | | 0.1 | 0/11 | – | 0/9 | – |

*The following code was used to conserve space in the table:
G — supernate from yeast cells ground with sand in a ball mill at 5° C.
$E_{50}$, $E_{67}$, $E_{75}$ — precipitate secured by adding 10 per cent sodium acetate, 1 per cent acetic acid and ethanol. The subscript numerals represent the percentage of ethanol used.
$S_{30}$, $S_{60}$ — precipitate obtained by adding ammonium sulfate to yield concentrations of 30 and 60 per cent, respectively.
Z — precipitate resulting from addition of 0.025 M zinc acetate.
†Dissolved in 0.1 ml. saline solution and injected intracutaneously.

## RESULTS

When infected rabbits were skin tested with somatic antigens isolated from yeast phase cells, there was no evidence of any fraction which possessed the desired properties of simultaneous high sensitivity and high specificity. These results are presented in Table I. In this table, as well as in the other tables, a reference fraction, Number 200, which had been isolated from the yeast phase supernate by ethanol precipitation, was included since it possessed the desired characteristics within a certain weight range.

TABLE II. SKIN TESTS WITH FRACTIONS PRECIPITATED FROM CULTURAL SUPERNATES WITH ETHANOL

| PERCENTAGE OF ETHANOL USED FOR PRECIPITATION | ANTIGEN NUMBER* | SKIN REACTIONS IN RABBITS | | | |
|---|---|---|---|---|---|
| | | *B. dermatitidis*-INFECTED | | *H. capsulatum*-INFECTED | |
| | | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) |
| 50 | 81 | 0/5 | – | 0/6 | – |
| | 119 | 0/10 | – | 5/10 | 3 |
| 67 | 83 | 0/5 | – | 6/6 | 6 |
| | 123 | 0/10 | – | 10/10 | 10 |
| | 124 | 0/10 | – | 10/10 | 10 |
| 71 | 200 | 0/11 | – | 9/9 | 8 |
| 75 | 84 | 0/5 | | 6/6 | 10 |
| | 125 | 0/10 | | 10/10 | 7 |
| | 126 | 0/10 | | 10/10 | 8 |
| 80 | 86 | 0/5 | | 5/6 | 8 |
| | 127 | 0/5 | | 3/10 | 7 |
| | 138 | 0/5 | | 11/11 | 11 |
| 83 | 129 | 0/10 | – | 0/10 | – |
| | 130 | 0/10 | – | 0/10 | – |
| | 142 | 0/9 | – | 7/10 | 9 |

*10 μg dissolved in 0.1 ml. of saline solution and injected intracutaneously.

In Table II results are presented which show the skin test reactivity of antigens precipitated from yeast phase cultural supernates with varying concentrations of ethanol. It will be seen that excellent reactivity was secured with fractions precipitated by 67, 71, and 75 per cent ethanol. Antigens precipitated by ethanol concentrations above and below this range were not sufficiently sensitive.

The comparison of commercial histoplasmin and Fraction 200 is presented in Table III. This assay was performed in guinea pigs and it is obvious that the guinea pig does not react with the same intensity as the rabbit. This may reflect a species difference or may be due to the fact that guinea pigs were infected by intraperitoneal administration of cells while rabbits were infected by the intravenous route. Commercial histoplasmin failed to produce a reaction in more than six out of seven *H. capsulatum*-infected guinea pigs even at a 1 to 10 dilution, and hence was judged to be insufficiently sensitive. The yeast phase Fraction 200 reacted with seven out of seven *H. capsulatum*-infected guinea pigs at the 100 μg level and failed to cross react in nine *B. dermatitidis*-infected animals.

TABLE III. COMPARATIVE TITRATION OF COMMERCIAL HISTOPLASMIN AND YEAST PHASE FRACTION 200 IN GUINEA PIGS

| ANTIGEN | CONCENTRATION | SKIN REACTIONS IN GUINEA PIGS | | | |
|---|---|---|---|---|---|
| | | *B. dermatitidis*-INFECTED | | *H. capsulatum*-INFECTED | |
| | | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) |
| Histoplasmin | 1/10 | 7/9 | 7 | 6/7 | 9 |
| | 1/100 | 1/9 | 7 | 4/7 | 6 |
| | 1/1000 | 0/9 | – | 0/7 | – |
| Fraction 200 | 100 μg | 0/9 | | 7/7 | 7 |
| | 10 μg | 0/9 | | 1/7 | 6 |
| | 1 μg | 0/9 | | 0/7 | – |

Another comparative assay between histoplasmin and Fraction 200 is shown in Table IV. This titration was done in rabbits using twofold increments in antigen concentration. The histoplasmin was neither completely sensitive nor completely specific. Fraction 200 was sensitive within a range of 8 to 64 μg and was specific over a portion of this range, and thus represents a considerable improvement over the commercial histoplasmin.

TABLE IV. COMPARATIVE TITRATIONS OF COMMERCIAL HISTOPLASMIN AND YEAST PHASE FRACTION 200 IN RABBITS

| ANTIGEN | CONCENTRATION | SKIN REACTIONS IN RABBITS | | | |
|---|---|---|---|---|---|
| | | *B. dermatitidis*-INFECTED | | *H. capsulatum*-INFECTED | |
| | | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) | REACTORS / TOTALS | AVERAGE DIAMETER OF INDURATION (MM.) |
| Histoplasmin | 1:40 | 6/10 | 5 | 8/10 | 9 |
| | 1:80 | 1/10 | – | 8/10 | 9 |
| | 1:160 | 0/10 | – | 2/10 | 7 |
| | 1:320 | 0/10 | – | 1/10 | 7 |
| | 1:640 | 0/10 | – | 0/10 | – |
| Fraction 200 | 64 μg | 5/10 | 9 | 10/10 | 14 |
| | 32 μg | 3/10 | 3 | 10/10 | 14 |
| | 16 μg | 0/10 | – | 10/10 | 8 |
| | 8 μg | 0/10 | – | 10/10 | 7 |
| | 4 μg | 0/10 | – | 4/10 | 5 |
| | 2 μg | 0/10 | – | 1/10 | 4 |
| | 1 μg | 0/10 | – | 0/10 | – |

Although only cross-reactions with *B. dermatitidis* were presented in the tables, it should be pointed out that Fraction 200 and other similar yeast phase antigens were also tested in animals infected with *Cryptococcus neoformans, Sporotrichum schenckii, Coccidioides immitis,* and *Candida albicans.* In none of the latter four species was cross-reaction observed up to the 100 μg level.

Skin tests were conducted between 5 and 27 weeks postinfection. Although the reactivity was slightly higher at the fourth and fifth weeks, decrease in reactivity during subsequent weeks was not great enough to be of significance.

Although the skin test antigen was originally thought to be polysaccharide, the extraction of aqueous solutions with chloroform in order to remove protein was also found to destroy the reactivity of the antigen. The polysaccharide which remained following this treatment appeared to be inert in the delayed skin reaction. This points up the need for additional chemical studies which are now in progress.

## DISCUSSION

The tendency of histoplasmin to cross-react when used for skin tests in individuals sensitive to fungi other than *H. capsulatum,* emphasizes the need for fractionation studies to secure antigens of increased specificity. At the same time such an antigen would have to retain a high degree of sensitivity, that is, it should produce a delayed reaction in all known positives tested.

Antigenic fractions fulfilling these criteria in part have been isolated in the present study. The most satisfactory preparations were precipitated from the supernatant fluid of yeast phase cultures by ethanol. Somatic antigens obtained from yeast cells by several procedures were insufficiently sensitive or else they were cross-reactive.

The most specific fractions, which were precipitated from cultural supernates with 67 to 75 per cent ethanol, still cross-reacted with *B. dermatitidis*-infected rabbits above the 32 $\mu$g level; hence, the degree of specificity is only relative. It is evident, however, that these antigens constitute an improvement over histoplasmin. No cross-reaction was obtained when the yeast phase antigens were tested in rabbits infected with *C. neoformans, C. albicans, S. schenckii,* or *C. immitis.* Since a procedure designed to remove protein destroyed the skin test reactivity of the yeast phase antigen, leaving behind an inert polysaccharide, it may be that the material reported here is still heterogeneous and that further purification would eliminate cross-reactivity. It may be that further work will reveal an isolation procedure better than precipitation with ethanol at 5° C.

In addition to its enhanced specificity, the yeast phase antigen was superior to histoplasmin with regard to sensitivity, reacting with ten out of ten animals at doses ranging between 8 and 64 $\mu$g. A further advantage of the yeast phase antigen is that it can be dispensed as a dry powder and standardized on a weight basis.

## SUMMARY

Antigenic fractions have been isolated from yeast phase cultures of *Histoplasma capsulatum* and tested for skin reactivity in infected guinea pigs and rabbits. The primary purpose of this study was to find an antigenic fraction which would not cross-react with related fungi and which, at the same time, retained a high degree of sensitivity. Somatic antigens extracted from yeast cells were not satisfactory from this standpoint; however, fractions precipitated from the cultural supernate with 67 to 75 per cent ethanol possessed high sensitivity and relatively good specificity. Although some cross-reaction was secured in animals infected with *Blastomyces dermatitidis,* the ethanol-precipitated antigens were superior to crude histoplasmin. No cross-reactions

were observed in animals infected with *Coccidioides immitis, Cryptococcus neoformans, Candida albicans,* or *Sporotrichum schenckii.* Although chemical studies on the yeast phase antigen have not been completed, a procedure designed to remove protein destroyed the skin test reactivity, leaving behind an inert polysaccharide. Further chemical studies are in progress.

REFERENCES

1. Emmons, C. W., Olson, B. J., and Eldridge, W. W.: Studies of the Role of Fungi in Pulmonary Disease. I. Cross Reactions With Histoplasmin, Pub. Health Rep. **60**: 1383, 1945.
2. Howell, A.: Studies on Fungus Antigens. I. Quantitative Studies of Cross Reactions Between Histoplasmin and Blastomycin in Guinea Pigs, Pub. Health Rep. **62**: 631, 1947.
3. Smith, C. E., and others: Histoplasmin Sensitivity and Coccidioidal Infections. I. Occurrence of Cross Reactions, Am. J. Pub. Health **39**: 722, 1949.
4. Furcolow, M. L., and Nelson, W. E.: Personal communication to C. E. Smith.
5. Cross, F. W., and Howell, A.: Studies of Fungus Antigens. II. Preliminary Report on the Isolation of an Immunologically Active Polysaccharide From Histoplasmin, Pub. Health Rep. **63**: 179, 1948.
6. Pates, A. L.: The Isolation of Antigens Formed by Histoplasma Capsulatum and Their Evaluation by Precipitin and Hypersensitivity Reactions, Thesis, University of Michigan, 1948.
7. Scheff, G. J.: Biochemical and Immunological Properties of Histoplasma Capsulatum, Yale J. Biol. & Med. **18**: 41, 1945.
8. Van Pernis, P. A., Benson, M. E., and Holinger, P. H.: Specific Cutaneous Reactions With Histoplasmosis, J. A. M. A. **117**: 436, 1941.
9. Dyson, J. E., and Evans, E. E.: Skin Test Antigens From Yeast Phase Cultures of Blastomyces Dermatitidis and Histoplasma Capsulatum, Univ. Michigan M. Bull. **20**: 53, 1954.
10. Sevag, M.: Eine Neue Physikalische Enteiweissungsmethode zur Darstellung Biologisch Wirksamer Substanzen. Isclierung von Kohlenhydraten aus Huhnereiweiss und Pneumococcen, Biohem. Ztschr. **273**: 419, 1934.

# THE EFFECTIVENESS OF ANTIBACTERIAL DRUGS UPON THE GRAM-NEGATIVE INTESTINAL FLORA

J. V. Martins Campos, M.D., J. Fernandes Pontes, M.D., W. Hoenen, M.D., and N. Kusminski, M.D.
São Paulo, Brazil

THE study of the reduction of the intestinal flora by antibacterial drugs is of great clinical and experimental significance. Prophylaxis for gastrointestinal surgery made it necessary to sterilize the intestinal tract. In 1940 Marshall and associates[1] were the first to perform clinical investigations with slowly absorbed compounds by using sulfanilylguanidine (sulfaguanidine) against the coliform flora. Black, McKibbin, and Elvehjem[2] did the first studies on animal growth in order to investigate the importance of the intestinal flora. Poth and associates[3, 4] described the theoretical requirements for the ideal intestinal antiseptic. However, Riddell[5] and Kirsner and associates,[6] after extensively reviewing the literature, concluded that none of the many investigated drugs fulfill those requirements, mostly due to early developing bacterial resistance and side effects.

Our studies were designed to establish a criterion for the selection of either a single drug or a combination of drugs most effective in producing a rapid, marked, and prolonged depression of the intestinal flora. Instead of trying to investigate the relationship between biochemical changes and the depression of the gastrointestinal flora by an antibacterial drug,[7] we proposed to limit our observations to the effect of several drugs and drug combinations upon the gram-negative flora. Although this group does not represent the entire bacterial population, it is a sufficiently significant part as far as number and biologic activity are concerned.

## MATERIAL AND METHODS

Fifty hospitalized adult patients were chosen for the experiments. None of them was found to suffer from any abnormality of the gastrointestinal tract. Preliminary bacteriologic investigations showed that their intestinal flora was normal and that they were not carriers of pathogenic germs of the enteric group. They were not receiving any medication which possibly could interfere with the investigation. No enemas or laxatives were used, and the patients were kept on a normal diet ad libitum. Only cases with one regular daily bowel movement before and during the experiment were included.

As indicated in Table I, 3 treatment groups were studied on 45 patients: antibiotics, nonabsorbable sulfa drugs, and combinations of both. Each drug or combination of drugs was administered orally to 5 patients in the dosage indicated in Table I. The period of treatment varied for each of the 3 treatment groups according to optimal results obtained in preliminary trials; however, dosage and periods of treatment were identical within each group.

From the Therapeutic Clinic (Professor Cantídio de Moura Campos), Department of Gastroenterology, of the "Hospital das Clínicas," Medical School of the University of São Paulo, Brazil.

Supported by a grant from Johnson & Johnson do Brasil, Research Department.
Received for publication April 22, 1954.

To count the gram-negative germs 1 Gm. of fresh feces was suspended in 100 ml. of saline and tenfold dilutions were plated (0.1 ml. inoculum) on Endo and E.-M.B. agar. In order to establish the *normal variation* between the bacterial counts of the same individual during the period of the investigation, as well as the *normal variation* between several individuals, 5 patients were left untreated and their fecal gram-negative flora was determined as in the treated cases.

Immediately before treatment 1 germ count was made for each of the 50 patients to establish the *basic count* of each individual case and therewith the average basic count of each group. This was followed by daily counts during the periods of treatment and by 1 or 2 counts after cessation of the treatment. The *activity indices* of a given treatment are shown by an increase or decrease of the gram-negative flora in these successive counts and are expressed as multiples or fractions of the individual basic count. The *mean activity index*

TABLE I. DOSAGE AND PERIOD OF TREATMENT USED FOR THE DIFFERENT DRUGS AND DRUG COMBINATIONS

| GROUP | TREATMENT | DOSAGE | PERIOD OF TREATMENT |
|---|---|---|---|
| I | Aureomycin | 0.50 Gm. every 6 hr. | 6 days |
| | Chloromycetin | 0.50 Gm. every 6 hr. | 6 days |
| | Terramycin | 0.50 Gm. every 6 hr. | 6 days |
| | Neomycin | 0.50 Gm. every 6 hr. | 6 days |
| II | Phthalylsulfathiazole | 0.25 Gm. per kg. body weight per 24 hr. | 8 days |
| | Succinylsulfathiazole | 0.25 Gm. per kg. body weight per 24 hr. | 8 days |
| III | Aureom. + Phthalyls. | In the above given dosages | 10 days |
| | Chlorom. + Phthalyls. | In the above given dosages | 10 days |
| | Neom. + Phthalyls. | In the above given dosages | 10 days |

(M.A.I.) is the mean arithmetic value of the activity indices of a given day of the 5 cases treated with the same drug or combination of drugs. The sequence of M.A.I.'s for each treatment, plotted as a curve (Figs. 1-6), takes into consideration the variations between days, showing the rapidity and persistence of action.

The *total activity index* (T.A.I.) is the mean arithmetic value of the activity indices of 1 patient during the period of treatment. The T.A.I.'s for the 5 patients treated identically, represented as bars (Fig. 1-6), take into consideration the variations between the cases, showing the uniformity of action.

The *general activity index* (G.A.I.) is obtained by averaging the mean activity indices and total activity indices obtained with the same drug or combination of drugs and by calculating the standard deviation. The G.A.I. with the standard deviations (Fig. 7) gives an indication of the over-all drug activity in all 5 cases throughout the whole period of treatment.

## RESULTS

The results are presented graphically in Figs. 1 to 7. The normal variations obtained by the daily bacterial counts of the feces of 5 untreated patients were ±0.52 for the mean activity index (M.A.I.), ±0.38 for the total activity index (T.A.I.), and ±0.96 for the general activity index (G.A.I.).

A. *Antibiotics.—Aureomycin* (Fig. 1, *A*) exhibited a rapid action, reaching its maximal activity after 1 day (M.A.I., −2.3). However, its reduction level was not well sustained, and during the treatment the bacterial counts reached the normal variation range. *Chloromycetin* (Fig. 1, *B*) exhibited a very similar effect except for a somewhat slower action with maximal ac-

tivity after 4 days (M.A.I., −2.6). Both drugs had a uniform action, lowering the bacterial counts significantly in all cases but one, in which Chloromycetin failed to do so. *Terramycin* (Fig. 2, *A*) not only failed to inhibit the gram-negative flora in 4 out of 5 cases, but the M.A.I. rose significantly above the normal variation range in the last 2 treatment days and for 6 days after the treatment. *Neomycin* (Fig. 2, *B*) had a slower and less marked action (M.A.I., −1.8) than Aureomycin and Chloromycetin, but this action was sustained until the end of the treatment, after which the counts rose above the normal variation range.

B. *Nonabsorbable Sulfa Drugs.*—Both Phthalylsulfathiazole (*Sulfathalidine*) (Fig. 3, *A*) and Succinylsulfathiazole (*Sulfasuxidine*) (Fig. 3, *B*) exhibited maximal activity only after 6 days. This inhibition, however, was quite marked (M.A.I., −3.2 and −2.6) and present in all 10 cases. During the last 2 days of treatment and the 2 posttreatment days the bacterial counts tended to increase and reached almost the normal level.

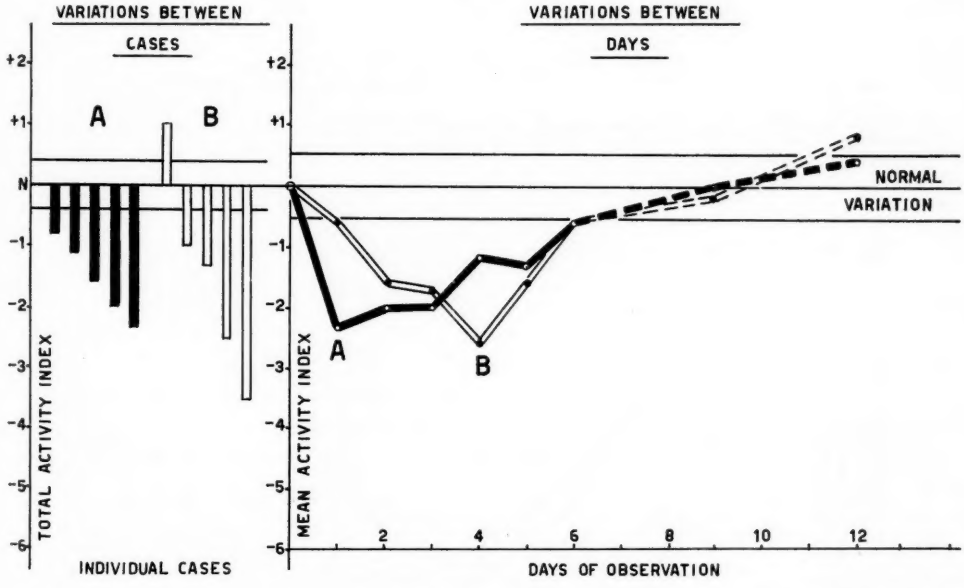



Fig. 1.—Effect of Aureomycin and Chloromycetin upon counts of the gram-negative fecal flora. The interrupted lines represent the posttreatment days. *A*, Aureomycin; *B*, Chloromycetin.

C. *Combined Treatments.*—The *combination of Sulfathalidine with Aureomycin* (Fig. 4) was characterized by its rapid action, producing a more marked reduction of the bacterial count (M.A.I., −5.1) than that achieved by any of the single drugs. This action was well sustained throughout the whole period of treatment and was present in all 5 cases treated. In contrast, *the combination of Sulfathalidine with Chloromycetin* (Fig. 5) showed essentially the same antibacterial effectiveness as the 2 single drugs. The *combination of Sulfathalidine with Neomycin* (Fig. 6) had a similarly rapid and uniform action as

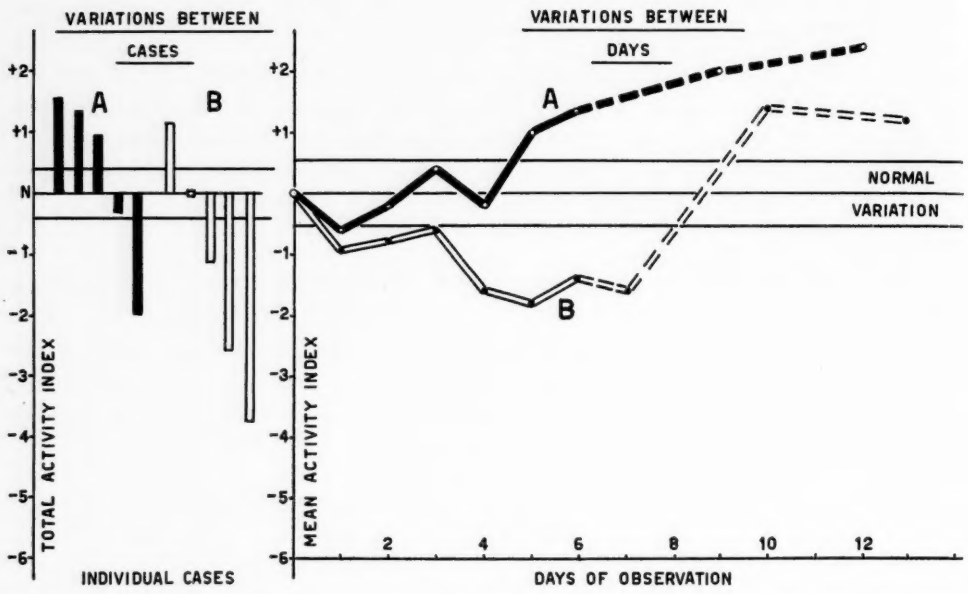



Fig. 2.—Effect of Terramycin and Neomycin upon counts of the gram-negative fecal flora. The interrupted lines represent the posttreatment days. *A*, Terramycin; *B*, Neomycin.

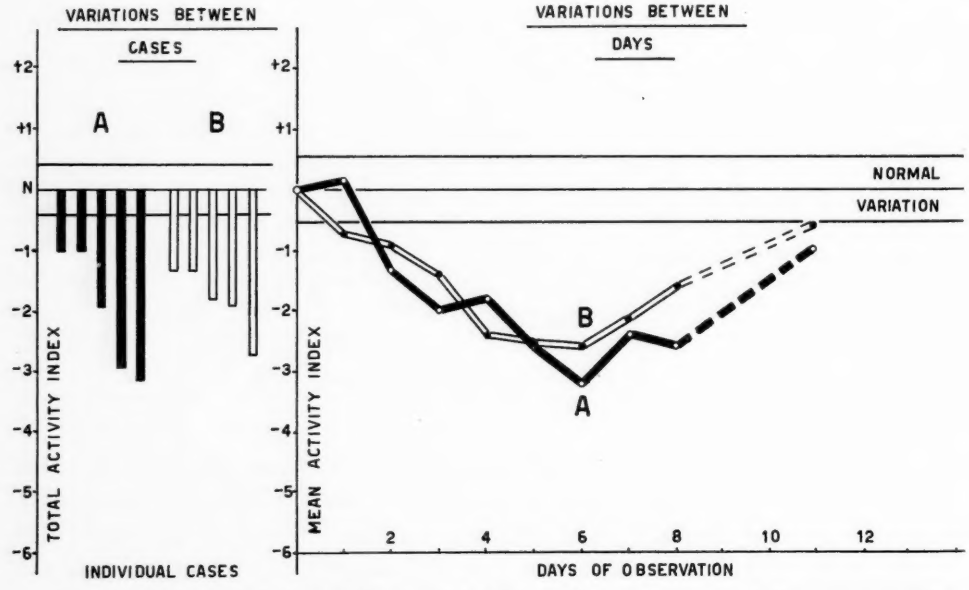



Fig. 3.—Effect of Sulfathalidine and Sulfasuxidine upon counts of the gram-negative fecal flora. The interrupted lines represent the posttreatment days. *A*, Phthalylsulfathiazole; *B*, Succinylsulfathiazole.

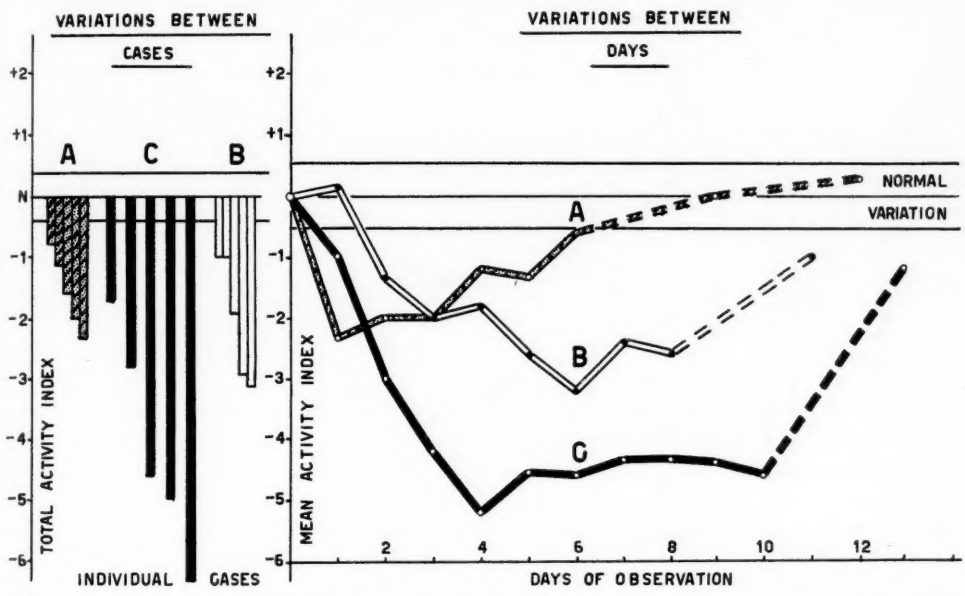



Fig. 4.—Effect of the combined treatment with Sulfathalidine and Aureomycin upon counts of the gram-negative fecal flora. The interrupted lines represent the posttreatment days. *A*, Aureom.; *B*, Phthalyls.; *C*, Comb. Treatment.

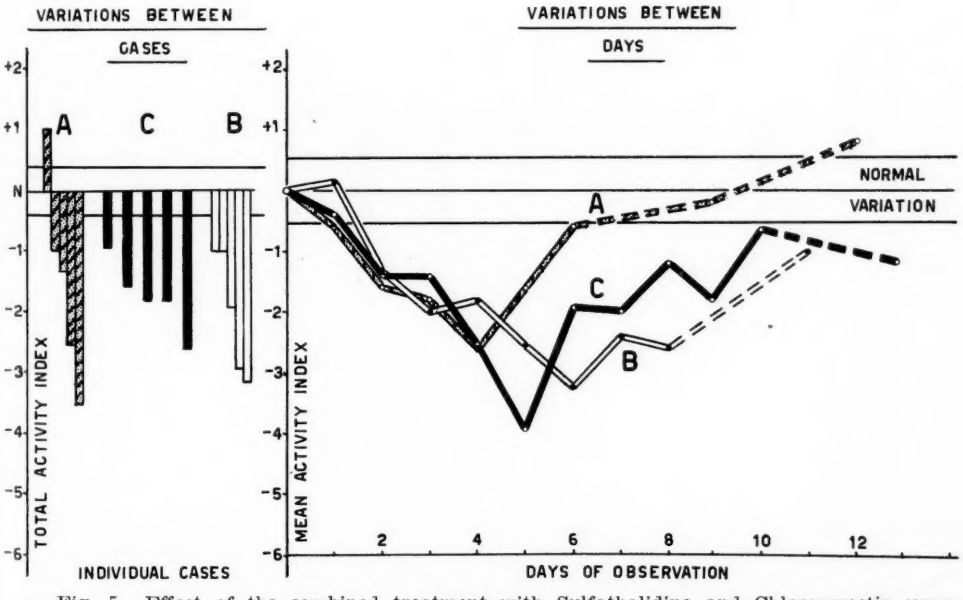



Fig. 5.—Effect of the combined treatment with Sulfathalidine and Chloromycetin upon counts of the gram-negative fecal flora. The interrupted lines represent the posttreatment days. *A*, Chlorom.; *B*, Phthalyls.; *C*, Comb. Treatment.

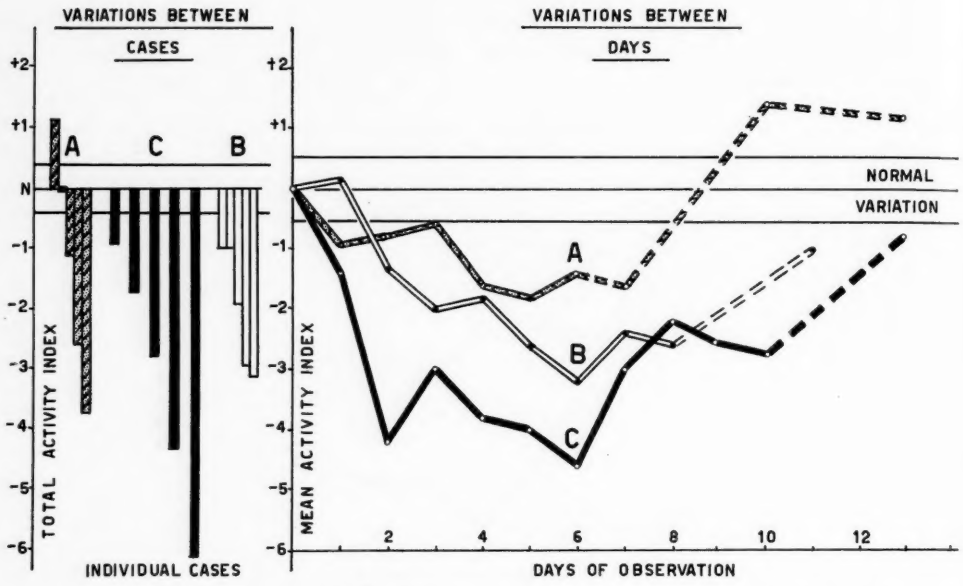



Fig. 6.—Effect of the combined treatment with Sulfathalidine and Neomycin upon counts of the gram-negative fecal flora. The interrupted lines represent the posttreatment days. *A*, Neom.; *B*, Phthalyls.; *C*, Comb. Treatment.

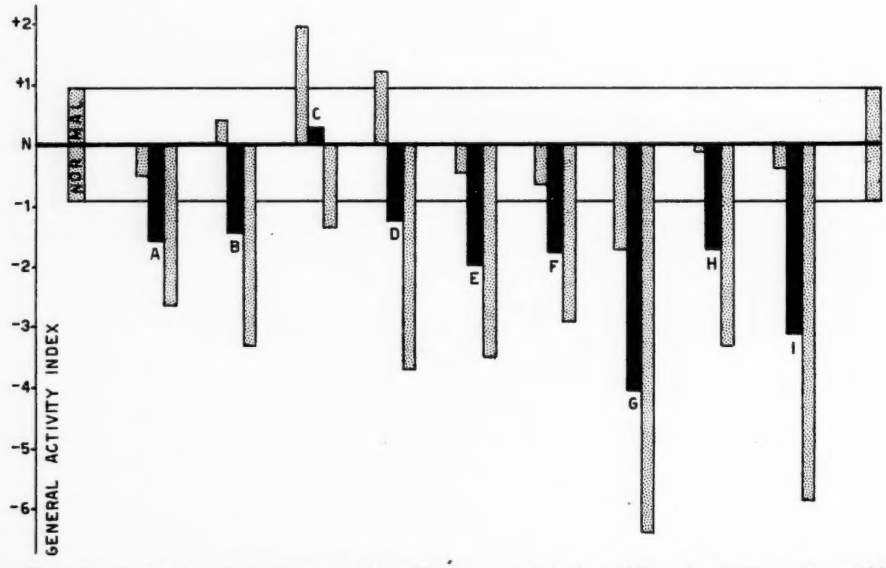



Fig. 7.—General comparison of the effectiveness of the different single and combined drug treatments. The dark columns represent the average depression of the gram-negative fecal flora, the light columns the possible spread after calculating the standard deviation. Antibiotics: *A*, Aureomycin; *B*, Chloromycetin; *C*, Terramycin; *D*, Neomycin. Sulfas: *E*, Phthalylsulfathiazole; *F*, Succinylsulfathiazole. Combined Treatments: *G*, Aureom. + Phthalyls.; *H*, Chlorom. + Phthalyls.; *I*, Neom. + Phthalyls.

the combination of the sulfa drug with Aureomycin. However, the effect was not as well sustained, and from the sixth day of treatment on, the bacterial counts increased toward pretreatment levels.

D. *General Comparison.*—A comparison of the general activity indices (Fig. 7) of the 9 types of treatment permits the conclusions that the single treatment by sulfa drugs seems to be more effective than the single treatment with the antibiotics used and that Sulfathalidine and Aureomycin or Neomycin combined reduce the gram-negative enteric flora more than any of the single treatments used. Terramycin, under these experimental conditions, is ineffective. The combination of Sulfathalidine with Chloromycetin does not enhance the effectiveness of either drug.

## DISCUSSION

Although there is considerable variation within each group, characteristic differences in the mode of action between the various drugs and drug combinations can be detected. Probably the sometimes erratic results are in part explained by variations in the mixed flora and therewith constantly varying sensitivity to the treatments. The tendency of the bacterial counts to return to the normal variation range during either the last days of treatment or the first posttreatment days is possibly explained by the outgrowing of drug-resistant forms after the sensitive bacteria have been mostly eliminated. This fact emphasizes the need to establish a time limit for useful employment of these antibacterial drugs, beyond which their use will be ineffective and might be even harmful.

Our results indicate that of the single drugs the sulfas have a more uniform, more sustained, and somewhat stronger, although slower, action than the antibiotics. Sulfathalidine in combination with Aureomycin constitutes the most effective treatment, producing the most rapid, deepest, and most sustained inhibition of the gram-negative intestinal flora.

The negative results obtained with 2 Gm. of Terramycin daily confirm the results of Rivera and Sborov,[8] who found that the lower limit of efficiency for this drug is 4 Gm. daily.

A complete eradication of the gram-negative flora is not achieved under our experimental conditions. Further investigations will be necessary to clarify the mode of action of combinations of antibacterial drugs, principally in dealing with mixed bacterial populations. The complexity of this problem was pointed out recently by Jawetz and Gunnison.[9]

## SUMMARY

Several antibiotics, nonabsorbable sulfa drugs, and combinations of both were investigated for their antibacterial action on the gram-negative intestinal flora; daily bacterial counts in feces were performed on 50 patients. Of the single drugs the sulfas exhibited a slower, but more marked, more sustained, and more uniform action than the antibiotics. The combination of Sulfathalidine with Aureomycin gave the most desirable results.

REFERENCES

1. Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, J. T., Jr.: Sulfanilylguanidine: A Chemotherapeutic Agent for Intestinal Infections, Bull. Johns Hopkins Hosp. **67**: 163, 1940.
2. Black, S., McKibbin, J. M., and Elvehjem, C. A.: Use of Sulfaguanidine in Nutrition Experiments, Proc. Soc. Exper. Biol. & Med. **47**: 308, 1941.
3. Poth, E. J., and Knotts, F. L.: Clinical Use of Succinylsulfathiazole, Arch. Surg. **44**: 208, 1942.
4. Poth, E. J., Fromm, S. M., Wise, R. I., and Hsiang, C. M.: Neomycin, a New Intestinal Antiseptic, Texas Rep. Biol. & Med. **8**: 353, 1950.
5. Riddell, M. I.: A Review of the Literature on Preoperative Prophylaxis of the Bowel With Antibacterial Agents, Am. J. M. Sc. **223**: 301, 1952.
6. Kirsner, J. B., Levin, E., and Palmer, W. L.: Use of Antibiotics in Gastrointestinal Diseases, Arch. Int. Med. **90**: 677, 1952.
7. Sborov, V. M., Sutherland, D. M., and Watson, C. J.: The Effect of Aureomycin on Urobilinogen Formation and the Fecal Flora, J. Lab. & Clin. Med. **37**: 52, 1951.
8. Rivera, J. A., and Sborov, V. M.: The Effect of Terramycin on the Intestinal Flora, Gastroenterology **17**: 546, 1951.
9. Jawetz, E., and Gunnison, J. B.: Antibiotic Synergism and Antagonism: An Assessment of the Problem, Pharmacol. Rev. **5**: 175, 1953.

# THE ERYTHROCYTE POTASSIUM CONCENTRATION IN CONDITIONS OF HYPONATREMIA AND HYPERNATREMIA

HARVEY C. KNOWLES, JR., M.D., GABRIELE ALVERSON, AND
EDWARD RUBENSTEIN, M.D.
CINCINNATI, OHIO

## INTRODUCTION

NUMEROUS observations have been reported concerning alterations in the potassium content of the erythrocyte. Although studies in vitro have revealed many injurious agents which may vary the concentration, such agents usually do not apply in clinical pathology. In 1942 Guest[1] found a decrease of potassium in the erythrocyte in diabetic acidosis and suggested that this decrease might reflect a deficit in total body stores. Since then, with the recent emphasis on potassium physiology, many investigators have attempted to show a direct relation between the potassium content of the erythrocyte and that of the whole organism. Despite considerable evidence accumulated to substantiate this relation, there is sufficient discord among findings to suggest that other factors may also influence the erythrocyte potassium content.

In rats rendered potassium deficient by diet, some investigators have reported a decreased erythrocyte content of potassium,[2, 3] while others have obtained equivocal results.[4] In clinical states of potassium deficiency both a decrease[5-7] and no change[8, 9] in erythrocyte concentration have been observed. Surveys of large groups of patients with varied disorders have tended to show a decreased erythrocyte potassium content when the serum concentration was low.[10, 11] However, review of the data of these surveys reveals many instances wherein the erythrocyte concentration of potassium was not in accord with the estimated body content as measured by clinical condition and serum potassium level. In tuberculous meningitis a normal erythrocyte content of potassium has been demonstrated in the face of a muscle deficit.[12]

In view of these inconsistencies, and because of our own inability to correlate erythrocyte and total body potassium content satisfactorily, we have reviewed our data for other factors which might also influence the erythrocyte

From the Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.

This investigation was supported by a grant (H-1445) from the National Heart Institute, United States Public Health Service.

Presented at the National Meeting of the American Federation of Clinical Research, Atlantic City, N. J., May 2, 1954.

Received for publication Oct. 27, 1954.

potassium concentration. We wish to present evidence suggesting that alteration of this concentration may, in part, be due to variation in tonicity of the extracellular fluid as measured by the serum sodium concentration.

## METHODS

Over a two-year period studies have been made of the electrolyte composition of erythrocytes in a wide variety of disorders. In order to investigate, by clinical approach, factors which might influence electrolyte concentration, observations were made for the most part on subjects with endocrine disorders, and with primary derangements of electrolyte and acid-base metabolism.

Studies were made on 70 subjects, classified in five groups according to serum sodium concentration. Hyponatremia was considered to be present when the serum sodium concentration was 134 meq. per liter or less, and hypernatremia when the concentration was 150 meq. per liter or more. The first group consisted of 13 normal subjects aged 21 to 37. The second group included 34 patients with disease, but with normal serum sodium concentration. Among this group were two patients considered to be potassium deficient by clinical estimation and lowered serum potassium concentration, one from prolonged vomiting, one from diabetic acidosis. In the third group were 16 patients with disease and asymptomatic hyponatremia which failed to respond to salt therapy. In this group were one patient with familial periodic paralysis and lowered serum potassium, three patients with elevated serum potassium due to renal failure, and one patient with elevated serum potassium in mild Addisonian crisis. In the fourth group five series of determinations were carried out on three patients with hyponatremia who showed satisfactory clinical and chemical response to salt therapy. Salt depletion developed in the natural course of chronic pyelonephritis in one patient and iatrogenically in the other two in the management of malignant hypertension and hepatic coma, respectively. For ease of identification, this latter group of hyponatremia is designated "depletion hyponatremia," and subjects of the third group are termed "adaption hyponatremic." In the fifth group were two patients with hypernatremia. One patient had mild elevation of the serum sodium due to insufficient water intake in the course of diabetes insipidus, and the other had unexplained marked hypernatremia persisting for several weeks after abdominal surgery.

Serum and blood sodium and potassium concentrations were determined using a Weichselbaum-Varney flame photometer. Whole blood was prepared by hemolysis with water and determinations were made directly in the photometer. Results obtained by this method agreed with those found by wet or dry ashing. Hematocrits were obtained in duplicate by centrifuging Wintrobe tubes at 5,000 r.p.m. for 30 minutes. No correction was made for trapped plasma. The values of erythrocyte potassium and sodium are expressed per liter of cells and were calculated from serum and whole blood values and the hematocrit.

## RESULTS

In Table I are given the values of serum sodium and erythrocyte sodium and potassium. In the group of normonatremia plus disease there were no alterations in the mean values of erythrocyte potassium and sodium. However, in the group of adaption hyponatremia the mean values of erythrocyte potassium (87.4 meq. per liter) and of potassium plus sodium (98.7 meq. per liter) were significantly below normal ($P < 0.01$). The mean value of erythrocyte sodium of 11.3 meq. per liter was probably significantly depressed ($P < 0.02$). In the group of depletion hyponatremia the values of erythrocyte po-

tassium and potassium plus sodium were below normal range in all five instances. In the two patients with hypernatremia, one with a serum sodium of 150 meq. per liter had an erythrocyte concentration of 100 meq. per liter, at the upper limits of normal range, and the other had a serum sodium of 171 meq. per liter with an erythrocyte potassium concentration of 119 meq. per liter. In no instance was an abnormally elevated erythrocyte sodium concentration found.

TABLE I. THE CONCENTRATIONS OF SERUM SODIUM AND ERYTHROCYTE POTASSIUM AND SODIUM

| GROUP | | SERUM Na (MEQ./L.) | ERYTHROCYTE K (MEQ./L.) | ERYTHROCYTE Na (MEQ./L.) | ERYTHROCYTE K + Na (MEQ./L.) |
|---|---|---|---|---|---|
| Normal (13) | Ave. | 141 | 94.3 | 16.8 | 111 |
| | Range | (137-144) | (86-102) | (9-25) | (104-126) |
| | S.D. | ±1.9 | ±4.6 | ±4.4 | ±5.7 |
| Normonatremia disease (34) | Ave. | 142 | 96.2 | 15.0 | 111 |
| | Range | (136-147) | (85-105) | (1-26) | (95-127) |
| | S.D. | ±2.8 | ±5.0 | ±6.6 | ±8.9 |
| Hyponatremia adaption (16) | Ave. | 127 | 87.4 | 11.3 | 98.7 |
| | Range | (117-134) | (80-97) | (1-23) | (90-112) |
| | S.D. | ±5.7 | ±4.7 | ±7.4 | ±6.5 |
| Hyponatremia depletion (5) | Ave. | 106 | 72.2 | 7.4 | 79.6 |
| | Range | (86-136) | (67-79) | (1-12) | (68-91) |
| Hypernatremia (2) | Ave. | 161 | 110 | 17.2 | 127 |
| | Range | (150-171) | (101-119) | (12-22) | (123-131) |

In Fig. 1 are shown the relations of the serum sodium and erythrocyte potassium concentrations. The group of normonatremia plus disease is omitted as the data overlie exactly those of the normals. The relation is linear and significant ($T = 9.73$). In Fig. 2 are shown in similar fashion the relations of the concentrations of serum sodium and erythrocyte potassium plus sodium. Again a significant relation is present ($T = 10.4$), emphasizing parallel changes in tonicity.

## DISCUSSION

From the foregoing it is evident that there is a relation between the serum sodium concentration and the potassium content of the erythrocyte. This relation was first noted by Hoffman and Jacobs,[13] who suggested that it might be a reflection of parallel changes of tonicity in the serum and cell. McCance,[14] in his studies on experimental salt depletion in normal man, observed a decrease in erythrocyte potassium in the presence of a lowered serum sodium concentration. In the data reported by Hutt[11] the erythrocyte potassium content was also frequently decreased in the presence of hyponatremia. Wyngaarden and associates[7] demonstrated a rise in erythrocyte potassium following administration of potassium in the course of potassium deficiency.[7] However, there was also a concomitant rise in serum sodium. Frequently, in potassium-deficient states, such as are seen in gastroenteric disorders, diabetic

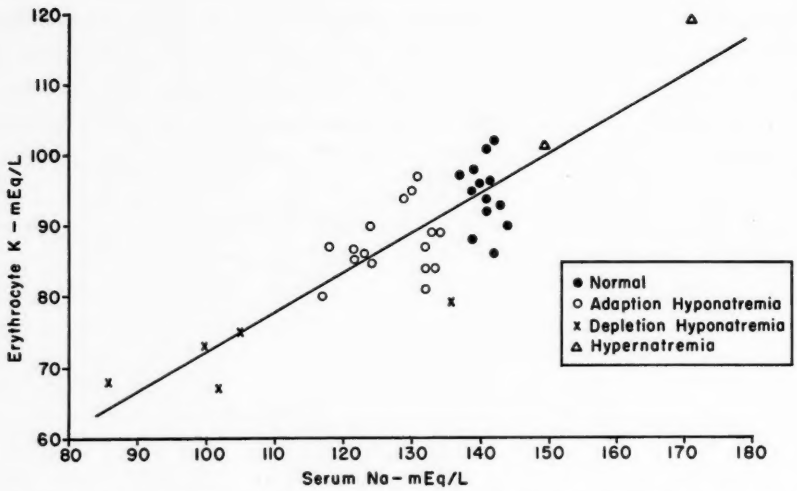



Fig. 1.—The relationship of serum sodium and erythrocyte potassium concentrations.

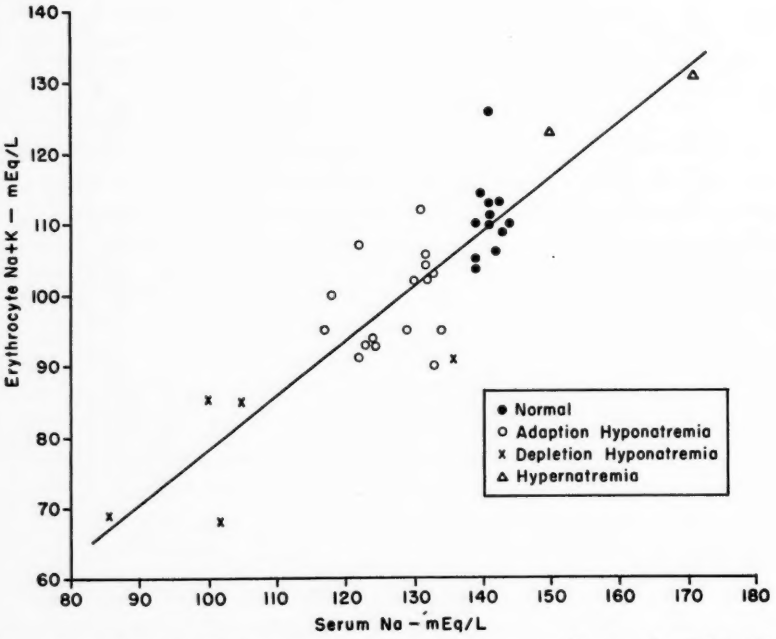



Fig. 2.—The relationship of the concentrations of serum sodium and erythrocyte potassium plus sodium.

acidosis, and renal failure, there is concurrence of hyponatremia which may, in part, account for the depression of the erythrocyte potassium content. Nevertheless, there are case reports of a lowered concentration of erythrocyte potassium in the face of normal serum sodium levels, and extreme potassium depletion in rats has resulted in a lowered erythrocyte content.

Ample evidence has accumulated that, in vitro, the osmolarity of the erythrocyte changes with alterations in environmental tonicity.[15] It is reasonable to assume that similar changes occur in vivo. With variations in extracellular tonicity, parallel changes in the erythrocyte could be brought about by alterations in water content, gain or loss of electrolytes, or changes in organic molecular dissociation occurring in cellular metabolism. This third factor may partially account for the wide range of erythrocyte cation concentrations found in the normal, but it seems unlikely that it could in itself fully account for the cellular osmotic changes necessary to equilibrate with significant changes in extracellular tonicity. Experiments to be reported suggest that although changes in water content are usually involved, in extreme conditions there may be actual gain or loss of potassium by the cell.[16]

Finally, the very low concentration of potassium in the erythrocytes of patients with depletion hyponatremia may be of some significance clinically. One is frequently faced with the dilemma of deciding when hyponatremia indicates a need for salt therapy. Although only five determinations were made in salt depletion, in all these determinations the erythrocyte content of potassium was below 80 meq. per liter. Admittedly, the serum sodium levels were markedly decreased also, but in one the concentration was 136 meq. per liter. More observations are needed to confirm this finding.

## SUMMARY

Studies made on the concentrations of the serum sodium and erythrocyte potassium in seventy subjects suggest that, in disease, alterations of the erythrocyte potassium content may be partly due to changes in extracellular fluid tonicity as measured by the serum sodium concentration.

## REFERENCES

1. Guest, G. M.: Organic Phosphates of the Blood and Mineral Metabolism in Diabetic Acidosis, Am. J. Dis. Child. **64:** 401-412, 1942.
2. Schwartz, R., Cohen, J., and Wallace, W. M.: Tissue Electrolyte Changes of the Whole Body, Muscle, Erythrocytes, and Plasma of Rats on a Potassium Deficient Diet, Am. J. Physiol. **172:** 1-6, 1953.
3. Spargo, B.: Kidney Changes in Hypokalemic Alkalosis in the Rat, J. Lab. & Clin. Med. **43:** 802-814, 1954.
4. Hegnauer, A. H.: The Effect of a Low Potassium Diet and of Desoxycorticosterone Acetate on the Cation Content of Rat Erythrocytes and Muscle, J. Biol. Chem. **150:** 353-357, 1943.
5. Nichols, G., Jr., and Nichols, N.: Electrolyte Equilibria in Erythrocytes During Diabetic Acidosis, J. Clin. Invest. **32:** 113-120, 1953.
6. Kennedy, T. J., Jr., Winkley, J. H., and Dunning, M. F.: Gastric Alkalosis With Hypokalemia, Am. J. Med. **6:** 790-794, 1949.
7. Wyngaarden, J. B., Keitel, H. G., and Isselbacher, K.: Potassium Depletion and Alkalosis: Their Association With Hypertension and Renal Insufficiency, New England J. Med. **250:** 597-600, 1954.

8. Black, D. A. K., and Milne, M. D.: Experimental Potassium Deficiency in Man, Clin. Sc. **11**: 397-415, 1952.

9. Schwartz, W. B., and Relman, A. S.: Metabolic and Renal Studies in Chronic Potassium Depletion Resulting From Overuse of Laxatives, J. Clin. Invest. **32**: 258-271, 1953.

10. Lans, H. S., Stein, I. F., Jr., and Meyer, K. A.: The Relation of Serum Potassium to Erythrocyte Potassium in Normal Subjects and Patients With Potassium Deficiency, Am. J. M. Sc. **223**: 65-74, 1952.

11. Hutt, M. P.: Effect of Disease on Erythrocyte and Plasma Potassium Concentrations, Am. J. M. Sc. **233**: 176-181, 1952.

12. Harrison, H. E., Finberg, L., and Fleishman, E.: Disturbances of Ionic Equilibrium of Intracellular and Extracellular Electrolytes in Patients With Tuberculous Meningitis, J. Clin. Invest. **31**: 300-308, 1952.

13. Hoffman, W. S., and Jacobs, H. R. D.: The Partition of Potassium Between the Serum and Corpuscles in Health and Disease, J. LAB. & CLIN. MED. **19**: 633-644, 1933.

14. McCance, R. A.: The Changes in the Plasma and Cells During Experimental Human Salt Deficiency, Biochem. J. **31**: 1278-1284, 1937.

15. Guest, G. M., and Wing, M.: Osmometric Behavior of Normal Human Erythrocytes, J. Clin. Invest. **21**: 257-262, 1942.

16. Agna, J. A., and Knowles, H. C., Jr.: Unpublished observations.

# LIFESPAN OF FETAL ERYTHROCYTES

JAMES W. HOLLINGSWORTH, CAPTAIN, MC, USA
WASHINGTON, D. C.

THE immediate neonatal period of the human infant is characterized by a change from macrocytic polycythemia to normocytic anemia. This change is accompanied by an increase in indirect bilirubin and apparent jaundice, an observation which has led many to the conclusion that there is an increased rate of red cell destruction during this period. An alternative or additional explanation of this jaundice is the well-known impairment of liver function in the newborn infant.[1]

Red cells of the newborn have been found to differ in some respects from those of normal adults. Wintrobe and Shumacker[2] demonstrated the presence of macrocytic erythrocytes in the blood of the infant at birth, and the rapid disappearance of these cells from the circulation. Hsia, Goldbloom, and Gellis[3] found an increase in mechanical fragility of the erythrocytes, most marked during the first day of life and falling to normal in five days. These authors suggested that the fragile cells were the macrocytes.

Because of the technical difficulty of performing erythrocyte survival studies in infants, little work has been done. Mollison has published two reports on the application to this problem of the Ashby technique of differential agglutination. In his first report Mollison[4] wrote that he had detected, in some instances, an increased rate of hemolysis during the first ten days after injection of cord blood, followed by a normal rate of destruction. He found that erythrocytes obtained from adults exhibited a normal survival in newborn infants. His results were said to be variable and only one curve was illustrated. The necessity of using another infant as the recipient and correction for plasma volume and rapid growth of the recipient made interpretation of the results difficult. Mollison's second report[5] contradicted the first. "The original contention that the percentage survival of placental cells, ten days after their transfusion, is distinctly less than that of adult cells, is not upheld." The difference between the two results is explained by pointing out that the first study was done with skin-prick blood, while the second was done with venous blood. In the illustration accompanying the second report no points for the Ashby study of adult red cells are shown, and the points representing the disappearance of placental red cells begin approximately ten days after the transfusion.

From the Department of Hematology, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.
Received for publication Aug. 23, 1954.

We have undertaken the study of survival of placental red cells by a different method, that of tagging the cells of cord blood with radioactive chromium ($Cr^{51}$) and transfusing them into normal adult volunteers.

## MATERIALS AND METHODS

Approximately 15 ml. of blood was collected at the time of delivery from the umbilical vein, after clamping of the cord, and was placed in a 50 ml. glass-stoppered centrifuge flask containing 5 ml. of sterile acid-citrate-dextrose solution. One hundred microcuries of $Na_2Cr^{51}O_4$ were then placed in the flask. After one hour of incubation at room temperature the cells were washed once with sterile saline solution and injected into normal adult male volunteers of compatible blood group. Samples were taken for counting after 15 minutes, and at approximately weekly intervals thereafter. Samples were counted in a well-type scintillation counter for sufficient time to allow a random sampling error of less than 5 per cent in most of the samples.

Aliquots of the cord blood were heparinized for routine examination by methods employed in our laboratory. Fetal hemoglobin was estimated by measuring the amount of hemoglobin that resisted alkaline denaturation using the method of Singer, Chernoff, and Singer.[6]

## RESULTS

The lifespan of the fetal erythrocytes is compared with that of erythrocytes from normal adults (Fig. 1). The normal range in our laboratory for the $Cr^{51}$ method was obtained by tagging cells from thirteen normal males and measuring autogenous survival. The biologic half life of 24 to 36 days in these subjects compares well with the results obtained by others using slightly different techniques.[7-9] Because of the difficulties encountered in keeping our recipients under observation for prolonged periods, the ideal control study of giving tagged erythrocytes from normal adults to the same recipients of fetal cells was not performed. Without this control it is possible to explain our results by assuming that all six of the apparently normal recipients had unsuspected, mild hemolytic diseases. This was considered unlikely and the control study seemed unnecessary.

All the survival curves of the newborn red cells were distinctly steeper than normal, with biologic half lives ranging from 15 to 23 days (Fig. 1). The actual lifespan of placental red cells is even less than their survival after transfusion would indicate. Mollison[5] has pointed out that the infant in utero trebles its blood volume during the 100 days before birth and, therefore, the number of young red cells in cord blood is disproportionately high. The survival after transfusion of such a population of red cells should be better than that of adult cells. Thus, even if the rate of hemolysis of transfused placental cells were the same as that of adult cells, one would have to conclude, as did Mollison, that the average lifespan of placental cells is less than 120 days.

The present studies indicate that the rate of destruction of placental red cells varied from one infant to another (Table I). It was thought that some clue as to the reason for the short lifespan might be gained from further comparisons of the red cells of these infants. It has been suggested that the macro-

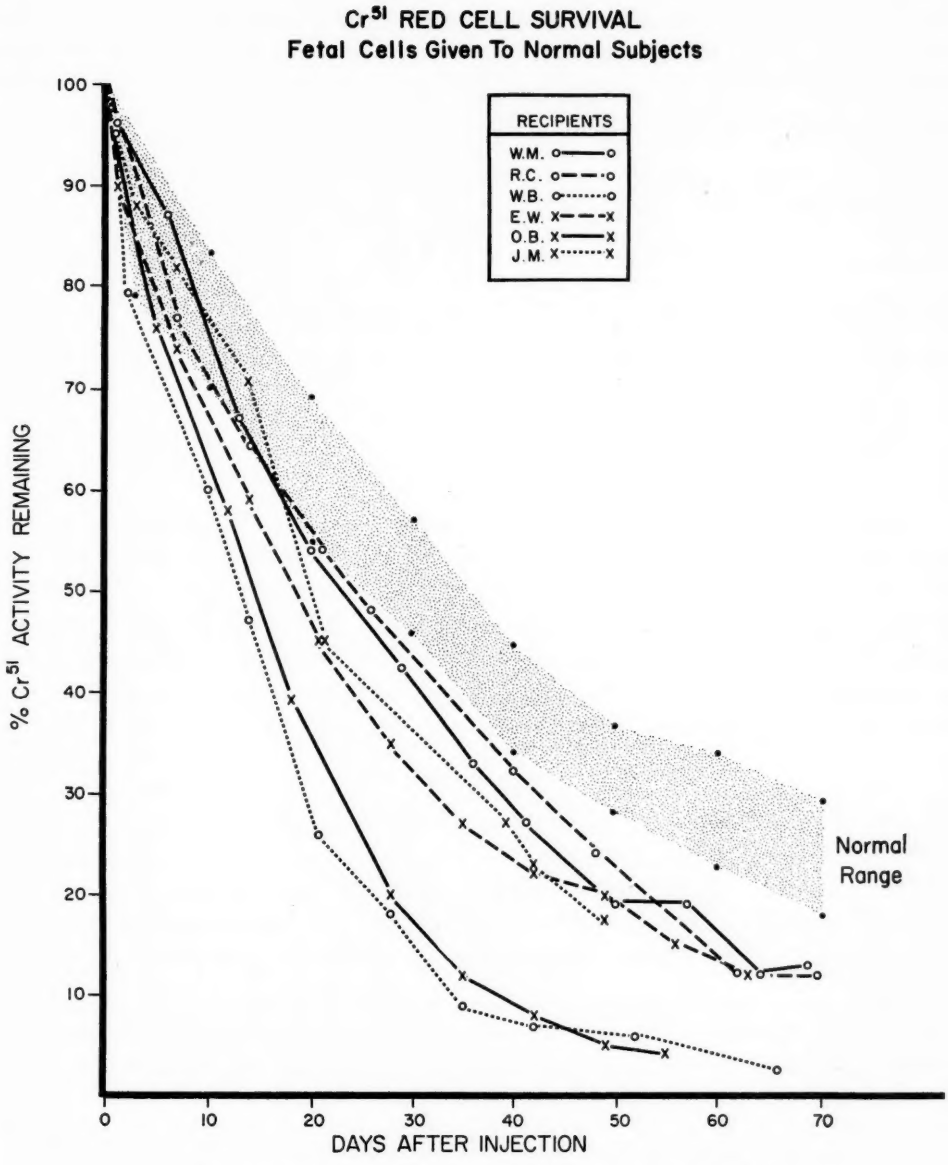



Fig. 1.—Survival of fetal erythrocytes in normal subjects.

cytic cells present at birth are the cells that are rapidly destroyed, but there is no correlation in our results between mean corpuscular volume (M.C.V.) and the rate of hemolysis (Table I). Nor do our data indicate that the amount of fetal hemoglobin is quantitatively related to the rate of hemolysis, since the five bloods studied fell within a narrow range of concentration (54 to 64 per cent). There was no correlation between the rate of hemolysis and the degree of plethora in these infants. The cause of short lifespan of placental red cells is not known.

TABLE I. STUDIES OF THE CORD BLOODS USED FOR TRANSFUSIONS

| RECIPIENT | R.B.C. ($\times 10^6$) | HGB. (GM.) | HEMATOCRIT (%) | M.C.V. CU. $\mu$ | RETIC. (%) | NUCLEATED R.B.C. (PER 100 W.B.C.) | FETAL HBG. (%) | HALF LIFE OF CR.51 (DAYS) |
|---|---|---|---|---|---|---|---|---|
| W. M. | 4.28 | 17.6 | — | — | 2.7 | 7 | — | 22 |
| R. C. | 5.10 | 18.1 | 58 | 114 | 4.1 | — | 58 | 23 |
| W. B. | 4.34 | 14.8 | 56 | 106 | 3.3 | 2 | 54 | 13 |
| E. W. | 6.1 | 16.1 | 52 | 85 | 8.6 | 7 | 58 | 18 |
| O. B. | 5.13 | 16.3 | 52 | 101 | 7.2 | 8 | 60 | 14 |
| J. M. | 5.22 | 12.1 | 50 | 96 | 8.4 | 6 | 64 | 19 |

Many investigators interested in the physiologic jaundice of early infancy have postulated that red cells formed under the hypoxic conditions in utero are unable to survive under conditions of normal oxygenation. There is evidence from studies of urobilinogen excretion that sudden hemolysis occurs after relief of hypoxia in high-altitude dwellers[10] and in children with congenital heart disease.[11] Studies of the survival of red cells formed under other conditions of hypoxia are in progress.[12]

## SUMMARY

Fetal erythrocytes, obtained from the umbilical cord at birth and tagged with radioactive sodium chromate, had a short lifespan when given to normal adult recipients. There was no evident correlation between rate of hemolysis and the amount of fetal hemoglobin, the degree of macrocytosis, or the total concentration of hemoglobin in the infant bloods that were studied.

The author wishes to express his appreciation to Dr. Cesar Reynafarje of Lima, Peru, for suggesting this study as a supplement to our work with subjects with high-altitude polycythemia.

## REFERENCES

1. Fashena, G. J.: Mechanism of Hyperbilirubinemia in Newborn Infants: Experimental Demonstration of Functional Hepatic Immaturity, Am. J. Dis. Child. **76:** 196, 1948.
2. Wintrobe, M. M., and Shumacker, H. B., Jr.: Comparison of Hematopoiesis in Fetus and During Recovery From Pernicious Anemia Together With Consideration of Relationship of Fetal Hematopoiesis to Macrocytic Anemia of Pregnancy and Anemia in Infants, J. Clin. Invest. **14:** 837, 1935.
3. Hsia, David Yi-Yung, Goldbloom, R. B., and Gellis, S.: Studies of the Mechanical Fragility of Erythrocytes. II. Relation to Physiologic Jaundice of the Newborn Infant, Pediatrics **13:** 24, 1954.

4. Mollison, P. L.: Physiologic Jaundice of the Newborn. Some New Measurements of the Factors Concerned, Lancet **1**: 513, 1948.

5. Mollison, P. L.: Blood Transfusion in Clinical Medicine, Springfield, 1954, Charles C Thomas, Publisher, pp. 108-111.

6. Singer, K., Chernoff, A. I., and Singer, L.: Studies on Abnormal Hemoglobins. I. Their Demonstration in Sickle Cell Anemia and Other Hematologic Disorders by Means of Alkali Denaturation, Blood **6**: 413, 1951.

7. Ebaugh, F. O., Jr., Emerson, C. P., and Ross, J. F.: The Use of Radioactive Chromium $^{51}$ as an Erythrocyte Tagging Agent for the Determination of Red Cell Survival in Vivo, J. Clin. Invest. **32**: 1260, 1953.

8. Weinstein, J. M., and LeRoy, G. V.: Radioactive Sodium Chromate for the Study of Survival of Red Blood Cells. II. The Rate of Hemolysis in Certain Hematologic Disorders, J. LAB. & CLIN. MED. **42**: 368, 1953.

9. Sutherland, D. A., McCall, M. S., Groves, M. T., and Muirhead, E. E.: The Survival of Human Erythrocytes Estimated by Means of Cells Tagged With Radioactive Chromium. A Study of the Normal State, J. LAB. & CLIN. MED. **43**: 717, 1954.

10. Merino, C. F.: Studies on Blood Formation and Destruction in the Polycythemia of High Altitude, Blood **5**: 1, 1950.

11. Josephs, H. W.: The Mechanism of the Reduction of Red Cells and Hemoglobin Following Operation for Tetralogy of Fallot, Bull. Johns Hopkins Hosp. **86**: 1, 1950.

12. Hollingsworth, J. W., Reynafarje, C., and Crosby, W. H.: Studies on Erythrocyte Lifespan in the Peruvian Andes. To be published.

# URINARY VITAMIN $B_{12}$ ACTIVITY FOLLOWING INTRANASAL ADMINISTRATION

RAYMOND W. MONTO, M.D., AND JAMES T. HOWELL, M.D.
DETROIT, MICH.

RECENT studies have demonstrated the effectiveness of the control of pernicious anemia by the administration of crystalline vitamin $B_{12}$ directly on the nasal mucosa.[1-3] With these observations in mind, it was deemed pertinent to study the absorption of the nasally instilled vitamin $B_{12}$ by determining the urinary excretion of the drug. Since it has been found previously that there is no significant difference in the urinary excretions of parenterally administered vitamin $B_{12}$ of normal persons or pernicious anemia patients, both types of subjects were used in this investigation.[4]

## MATERIALS AND METHODS

Two normal subjects were given nasal instillation of 200, 100, and 50 $\mu$g of crystalline vitamin $B_{12}$ in 0.5 ml. saline, each instillation on a separate day. In addition, two patients with classical manifestations of pernicious anemia in relapse received, under direct observation upon the nasal mucosa, 200 $\mu$g and 150 $\mu$g, respectively, of vitamin $B_{12}$ crystals. Urine specimens were collected for a two-hour period before and at two, five, and eight hours after the administration of the drug and assayed for vitamin $B_{12}$ activity utilizing the test organism *Lactobacillus leichmannii*.

## RESULTS

Figs. 1, 2, and 3 illustrate the results obtained in the two normal subjects. In the eight-hour test period, significant amounts of vitamin $B_{12}$ urinary activity were detected. This occurred in greater portions during the first two-hour period following administration. The $B_{12}$ activity in the urine appeared in amounts proportional to the quantity given nasally. Fig. 4 illustrates similar results obtained when 150 $\mu$g of crystalline $B_{12}$ was placed directly on the nasal mucosa of a patient with pernicious anemia in severe relapse. The subject was found to have large numbers of nasal polyps and severe chronic hypertrophy of the nasal mucous membranes. This patient, whose original erythrocyte count was 2.25 million, demonstrated a maximum reticulocytosis of 44.3 per cent on the sixth day following therapy. A complete hematologic remission was obtained for over a three-month period, after which the development of mild glossitis occurred. In a second subject (Fig. 5), with pernicious anemia in severe relapse, 200 $\mu$g of $B_{12}$ crystals was placed upon the nasal mucous membrane. Assay of the urine in this patient demonstrated the excretion of 12 per cent of the administered dose. This patient, with an initial erythrocyte count of 2.01 million, also produced a maximum reticulocytosis of 43.4 per cent on the seventh day after therapy and obtained a satisfactory clinical remission from the single administration of the drug.

From the Division of Hematology, Henry Ford Hospital, Detroit 2, Michigan.
Received for publication Sept. 8, 1953.

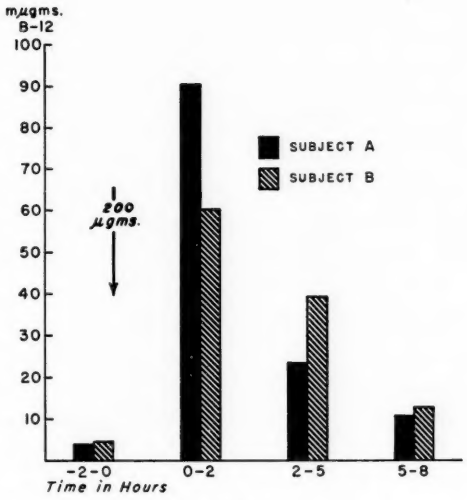



Fig. 1.

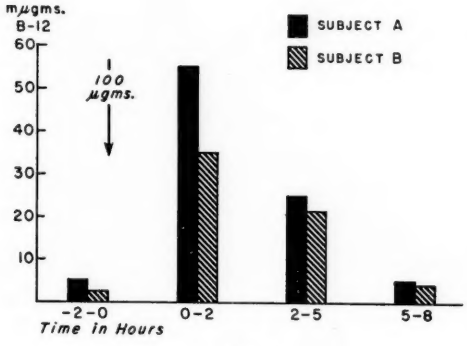



Fig. 2.

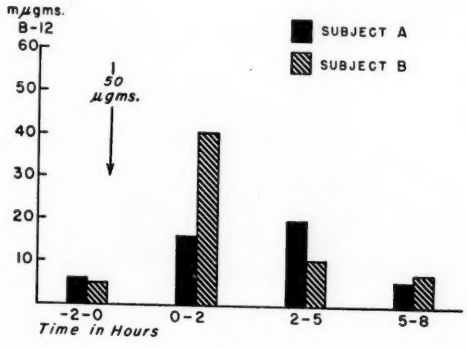



Fig. 3.

Figs. 1-3.—Urinary excretion of vitamin $B_{12}$ following its intranasal instillation in saline solution.

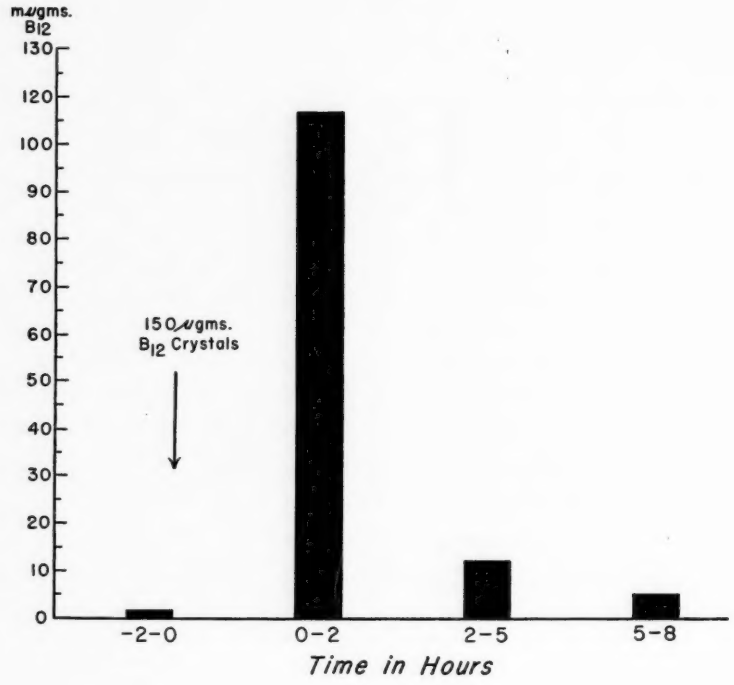



Fig. 4.

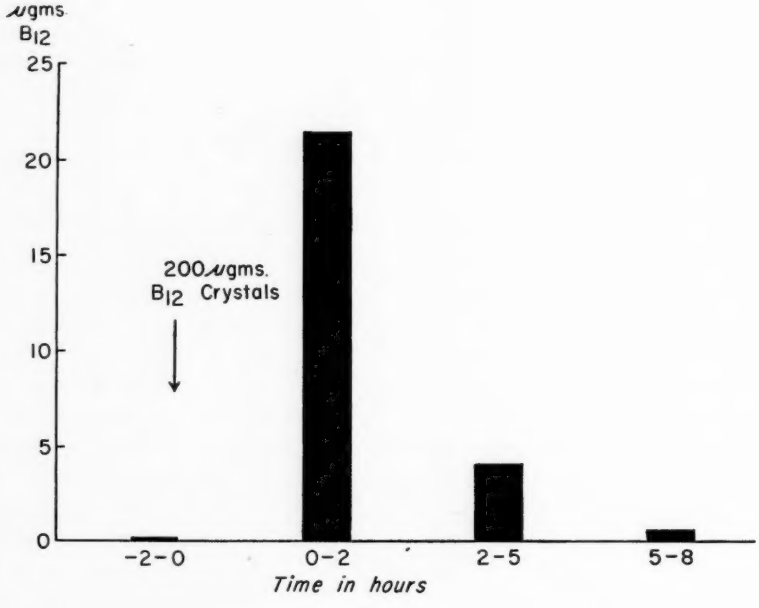



Fig. 5.

**Figs. 4 and 5.—Urinary excretion of vitamin $B_{12}$ after its intranasal topical application in crystalline form.**

## DISCUSSION

When vitamin $B_{12}$ is administered orally in the amounts utilized in this study, significant amounts cannot be recovered in the urine in either normal subjects or patients with pernicious anemia. Utilizing certain refined techniques, however, will demonstrate the $B_{12}$ in the serum of such patients in a "bound form."[5] Usually no urinary excretion of vitamin $B_{12}$ is detectable except when it is "flushed" out by large parenteral doses.[6] When given parenterally, the vitamin $B_{12}$ appears both in the blood and in the urine, largely as the free drug. The data presented here would suggest, therefore, that the vitamin $B_{12}$ is absorbed directly by the nasal mucosa and that the urinary excretion patterns are similar to those obtained by parenteral injection. These suppositions are further supported by a single experiment wherein nasal washings from a normal subject after the nasal administration of vitamin $B_{12}$ failed to produce a reticulocyte response when introduced into the stomach of a pernicious anemia patient in relapse.

It was observed that crystalline vitamin $B_{12}$ crystals in amounts of 200 and 150 $\mu$g produced no irritation of the nasal mucous membranes. Chronic disease of the nasal mucous membranes apparently does not impede absorption of vitamin $B_{12}$.

The authors wish to express appreciation to H. W. Schoenlein, Director of Bacteriological Laboratories, Difco Laboratories Incorporated, for the urinary $B_{12}$ determinations.

## REFERENCES

1. Monto, R. W., Rebuck, J. W., and Brennan, M. J.: Crystalline $B_{12}$ Inhalation Therapy in Pernicious Anemia, Am. J. M. Sc. **225**: 113, 1953.
2. Monto, R. W., and Rebuck, J. W.: Nasal Instillation and Inhalation of Crystalline Vitamin $B_{12}$ in Pernicious Anemia, Arch. Int. Med. **93**: 219, 1954.
3. Israels, M. C. G., and Shubert, S.: The Treatment of Pernicious Anaemia by Insufflation of Vitamin $B_{12}$, Lancet **1**: 341, 1954.
4. Conley, C. L., Krevans, J. R., Chow, B. F., Barrows, C., and Long, C. A.: Observations on the Absorption, Utilization, and Excretion of $B_{12}$, J. LAB. & CLIN. MED. **38**: 84, 1951.
5. Mollin, D. L., and Ross, G. I. M.: Vitamin $B_{12}$ Concentrations of Serum and Urine in the First 72 Hours After Intramuscular Injection of the Vitamin, J. Clin. Path. **6**: 54, 1953.
6. Schilling, R. F.: Intrinsic Factor Studies. II. Effect of Gastric Juice on the Urinary Excretion of Radioactivity After Oral Administration of Radioactive Vitamin $B_{12}$, J. LAB. & CLIN. MED. **42**: 860, 1953.

# PROBENECID AND SALICYLATES: THE QUESTION OF INTERACTION IN TERMS OF PENICILLIN EXCRETION

WILLIAM P. BOGER, M.D., S. CLYDE STRICKLAND, M.D., GILBERT M. BAYNE, M.D., AND JULINA GYLFE, M.D.
NORRISTOWN, PA.

PROBENECID (p-[Di-n-propylsulfamyl]-benzoic acid)* has been shown to exert certain inhibitory and reversible effects upon the renal tubules both in animals and in man.[1-3] From the standpoint of their clinical implication, the most interesting of these observations are the enhancement of penicillin plasma concentrations (penicillemia) by two to ten times, regardless of the type of penicillin or the route of administration employed, and the inhibition of renal tubular reabsorption of uric acid in gouty patients with consequent lowering of serum uric acid and an increase in the amount of uric acid excreted into the urine. Advantage has been taken of the first of these effects of probenecid in extending the application of orally administered penicillin to the handling of easily treated infections and to the intensifying of parenteral penicillin therapy of "resistant" infections, notably, subacute bacterial endocarditis. The uricosuric property of probenecid has been applied to the treatment of chronic gout, and by reason of its continuing effect upon the renal excretion of uric acid, the miscible uric acid pool can be significantly reduced toward normal[4, 5] and certain tophaceous deposits can be made to regress and even to disappear.[6-11]

Salicylates exert a marked uricosuric effect if administered in large daily doses (4 to 5 Gm.) and have, in addition, analgesic properties. Consequently, the salicylates enjoy a time-honored place in the therapy of chronic gout. It is understandable that probenecid, being better tolerated and having an equal or greater uricosuric effect than the salicylates *but no pain-relieving properties,* should have been used in combination with salicylates for the treatment of patients with gout. Such a combined use of salicylates and probenecid led to an interesting and completely unexpected observation. The uricosuric effects which are manifest when either agent is used alone are negated when these agents are employed concurrently.[5, 7, 10, 12] These findings led to the interdiction of concomitant use of these two substances.

Under these circumstances the questions arise whether, when salicylates and probenecid are coadministered, there is (a) interference with the antipyretic effects of salicylates, (b) mitigation of the analgesic properties of the

From the Department of Research Therapeutics, Norristown State Hospital, Norristown, Pa.
Received for publication Sept. 16, 1954.
*Probenecid is supplied under the trademark "Benemid" by Sharp and Dohme Division of Merck & Company, Inc.

salicylates, or (c) negation of the inhibitory effects of probenecid on the renal tubular secretion of penicillin. The first two questions have not been answered by suitably controlled studies, but extensive clinical experience has thus far failed to reveal evidence of interference with either the antipyretic or analgesic effects of salicylates by probenecid. In an effort to answer the question of interference of salicylates with the probenecid effect on penicillin, this study was undertaken.

## MATERIALS AND METHODS

*Patients.*—Six adult individuals, two males and four females, ranging in age from 26 to 48 years and without laboratory or physical evidence of cardiac, hepatic, or renal impairment, were selected for investigation.

*Plan of Study.*—Intravenously administered penicillin disappears at a rapid and predictable rate from the circulation and serves as an excellent indication of the degree to which renal tubular secretion of the antibiotic is inhibited by a variety of agents. Accordingly, in order that penicillin might be most purposefully employed as a reference substance, an intravenous dose of 500,000 units of sodium penicillin G was arbitrarily selected for the purposes of this study. Each of the six patients served as his own control during four phases of study, following (A) the intravenous administration of penicillin alone, (B) premedication with salicylates (acetylsalicylic acid) for a period of 24 hours and then penicillin intravenously, (C) coadministration orally of salicylates and probenecid for 24 hours and then penicillin intravenously, and (D) probenecid premedication for 24 hours and then penicillin intravenously. Phases (A), (B), and (C) were sequential, whereas a period of seven days intervened between Phases (C) and (D) in order that the circulation might be cleared of salicylates and probenecid. The six patients were studied in two groups of three each, a time interval of 1 month elapsing between the study of the first and second series of three patients. The protocol followed for the study of each patient was as follows. At 0 hour, 500,000 units of penicillin G was injected intravenously, and blood samples for penicillin assay were drawn at 1, 3, and 5 hours after injection. At 6, 12, 18, and 24 hours, each individual received 1 Gm. (15 gr.) of acetylsalicylic acid. At 24 hours, 500,000 units of sodium penicillin G was again injected intravenously. At 24, 25, 27, and 29 hours, blood specimens were again drawn and submitted for both salicylate estimations and penicillin assay. At 30, 36, 42, and 48 hours, the patients received concomitantly 1 Gm. (15 gr.) of acetylsalicylic acid and 0.5 gm. of probenecid. At 48 hours, 500,000 units of sodium penicillin G was administered intravenously and blood specimens were drawn at 48, 49, 51, and 58 hours. These specimens were submitted for salicylate and probenecid estimations and for penicillin assay. A period of 7 days was allowed to elapse before each patient was again premedicated with probenecid in a dose of 0.5 Gm. at 6-hour intervals for 24 hours and given 500,000 units of penicillin G intravenously. Blood specimens drawn at 24, 25, 27, and 29 hours were submitted for probenecid estimations and penicillin assays.

*Methods.*—The same batches of sodium penicillin G, acetylsalicylic acid, and probenecid were employed throughout the study. Penicillin plasma concentrations were determined by the *Sarcina lutea* cup-plate method,[13] salicylate plasma concentrations were determined by the method of Brodie and associates,[14] and probenecid determinations by the methylene blue method.[15]

## RESULTS

In Fig. 1 are presented both the individual and the average penicillemia values for the six patients studied in Phases (A) and (B). Salicylates in the doses that were administered had no significant effect upon the penicillemia in these subjects. The average values in Phase (A) were 2.84, 0.28, and 0.082 $\mu$

per milliliter at 1, 3, and 5 hours, respectively, and were not significantly different from the average figures for Phase (B), which were 2.81, 0.46, and 0.158 $\mu$ per milliliter at 1, 3, and 5 hours, respectively.

In Fig. 2 are presented the individual and average data for six patients studied in Phases (C) and (D). There was no apparent effect of salicylate upon the probenecid enhancement of penicillemia. The average figures in Phase (C) were 10.01, 1.73, and 0.78 $\mu$ per milliliter at 1, 3, and 5 hours, respectively, and in Phase (D), 10.30, 2.28, and 0.90 $\mu$ per milliliter at 1, 3, and 5 hours, respectively.

In Fig. 3 are presented the individual and average values for the salicylate plasma concentrations during Phases (B) and (C). The salicylemia attained after the administration of 4 Gm. of salicylate (1 Gm. every 6 hours) represented a "plateau" as indicated by the salicylate plasma values of 9.6, 9.5, 9.4, and 8.8 mg. per milliliter at 24, 25, 27, and 29 hours, respectively. These average values are not appreciably different from the plasma concentrations observed in Phase (C), which were 9.6 10.1, 10.3, and 9.2 mg. per 100 ml. at 48, 49, 51, and 53 hours, respectively.

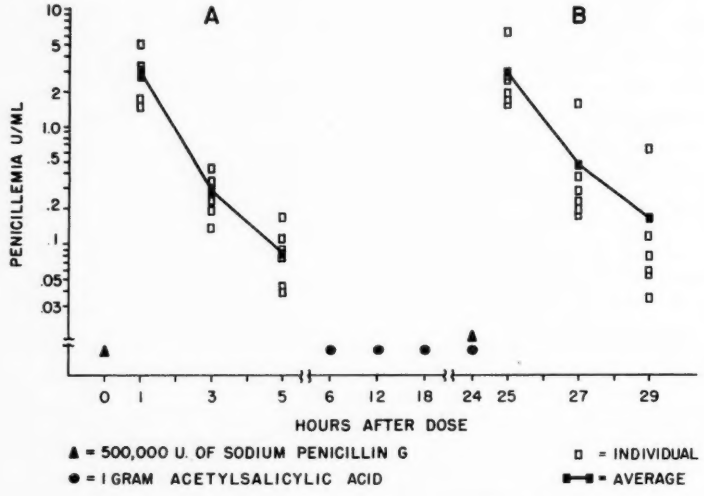



Fig. 1.—Salicylate effect on penicillemia, six patients.

Fig. 4 shows that the probenecid concentrations obtained during Phases (C) and (D) were in good agreement. In Phase (C) the levels were 5.2, 5.9, 5.7, and 4.8 mg. per 100 ml. at 48, 49, 51, and 53 hours, respectively, and in Phase (D), 7.02, 7.16, 6.9, and 8.16 mg. per 100 ml. at 24, 25, 27, and 29 hours after one day's premedication with probenecid.

In Fig. 5 are presented the average penicillemias* observed in six patients during the four phases of study. It is apparent that salicylates per se have exerted no influence upon penicillemia. Similarly, salicylates have neither added to nor detracted from the penicillin enhancement effect of probenecid.

*A statistical analysis was made of these data. For statistical reasons levels $<0.03$ were considered as 0.01 and all levels were transformed to $Y = \log$ (100 times the level).

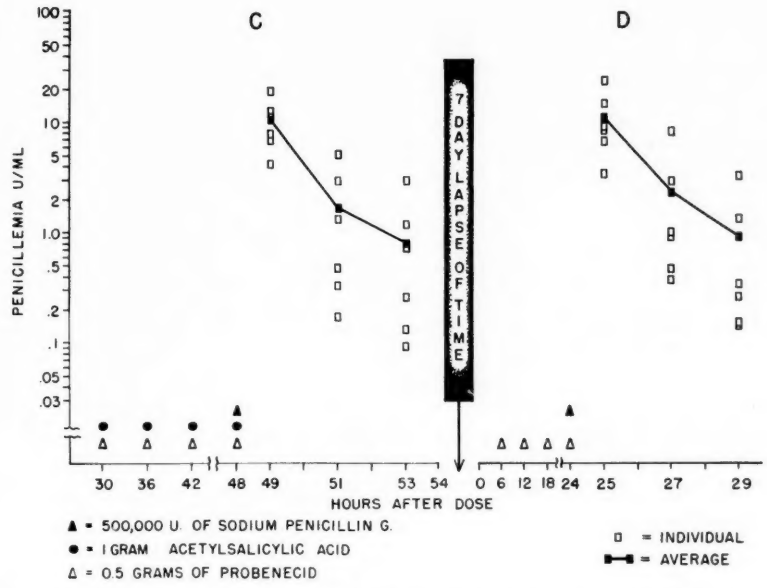



Fig. 2.—Probenecid enhancement of penicillemia with and without salicylates, six patients.

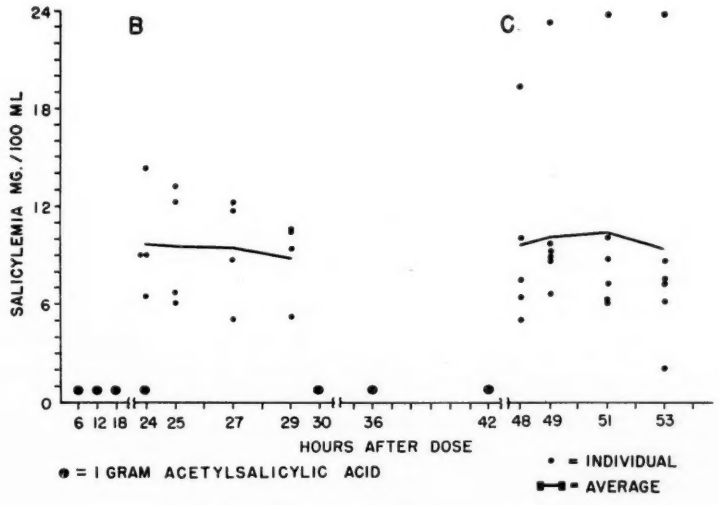



Fig. 3.—Salicylate plasma levels, six patients.

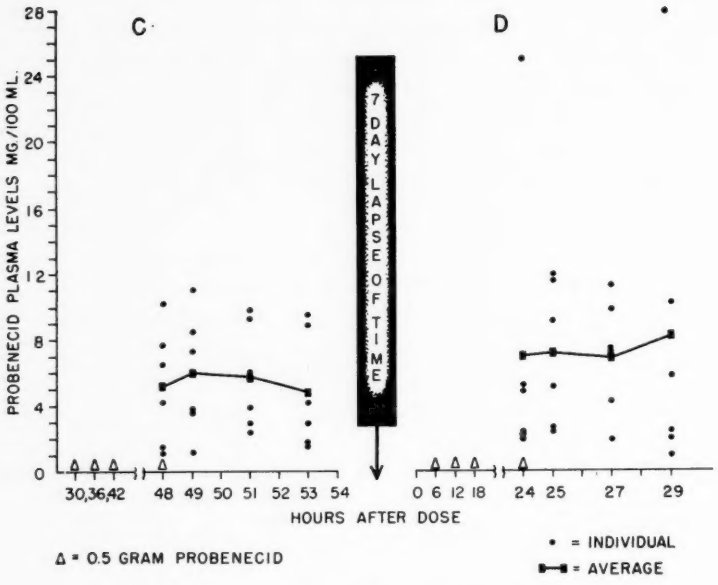



Fig. 4.—Probenecid plasma levels, six patients.

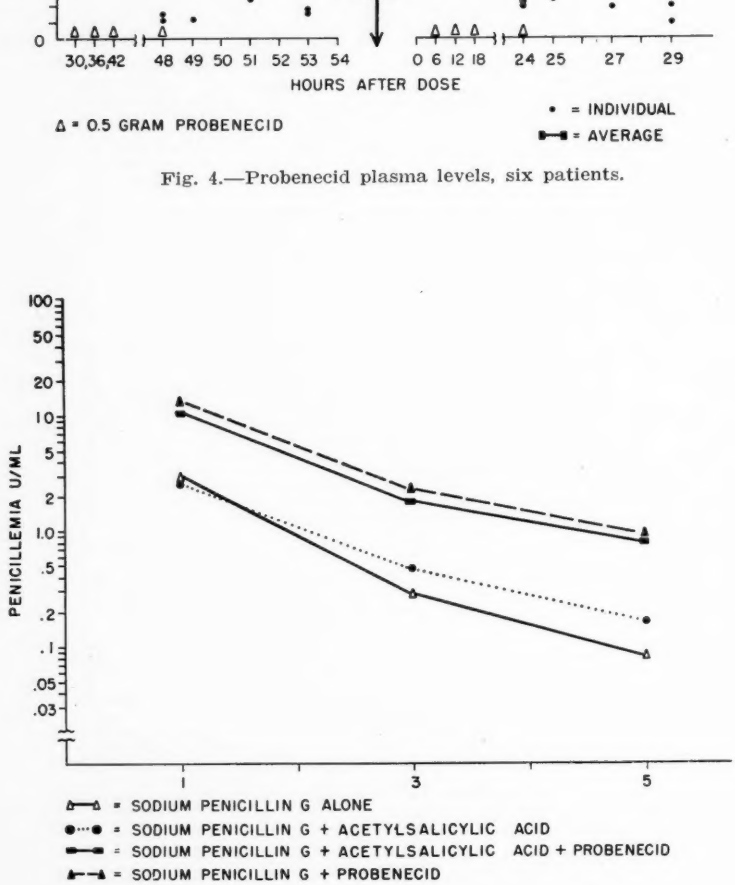



Fig. 5.—Penicillemia Phases A, B, C, and D averages, six patients.

| MEAN TRANSFORMED LEVELS | | | |
|---|---|---|---|
| | HOURS AFTER INJECTION | | |
| TREATMENT | 1 | 3 | 5 |
| A | 2.42 | 1.42 | 0.89 |
| B | 2.40 | 1.51 | 0.96 |
| C | 2.95 | 1.96 | 1.64 |
| D | 2.95 | 2.09 | 1.65 |

| "p" VALUE | LEAST SIGNIFICANT DIFFERENCES |
|---|---|
| 0.05 | 0.26 |
| 0.02 | 0.32 |
| 0.01 | 0.36 |
| 0.001 | 0.49 |

From the table of mean transformed values and the table of least significant differences, it is apparent that the "p' value is substantially greater than 0.05 regardless of the time period selected for the comparison of penicillin with penicillin plus aspirin and also for the comparison of penicillin and probenecid with penicillin and probenecid plus aspirin.

## DISCUSSION

In previous communications[3, 16] it has been pointed out that the plasma concentrations of probenecid that produce full inhibition of renal tubular excretion of penicillin fall in the range of 4 to 11 mg. per 100 ml., and that this range of concentration is observed regularly when 2 Gm. of probenecid is administered in divided doses over a 24-hour period, either as 1 Gm. every 12 hours or 0.5 Gm. every 6 hours. The observations recorded in this study are in agreement with the previously published findings in showing that the concentrations of probenecid were comparable during Phases (C) and (D) on a dose of 2 Gm. per day and that at plasma concentrations in excess of 4 mg. per 100 ml. there was full enhancement effect upon penicillin plasma concentration ($p < 0.001$ in this study).

The negation of uricosuric effects when salicylates and probenecid are coadministered has been confirmed by a number of investigators,[6-11, 17] but more recently it has been indicated that a salicylate concentration approximating 10 mg. per 100 ml. is critical in producing the reversal of the uricosuric effects of probenecid.[18] This study demonstrates that comparable salicylate concentrations do not negate the penicillin-enhancing effects of probenecid.

The close agreement of the salicylate plasma concentrations observed in Phases (B) and (C) confirms previous observations [19, 20] that probenecid in effective concentrations does not exert a significant effect upon the plasma concentrations of salicylates.

## SUMMARY

On the basis of a carefully controlled study of six patients it is concluded: (a) Acetylsalicylic acid in a daily dose of 4 Gm., resulting in an average salicylemia approximating 10 mg. per 100 ml. has no enhancement effect upon the penicillemia resulting from the intravenous administration of 500,000 units of sodium penicillin G. (b) When acetylsalicylic acid is administered orally in a daily dose of 4 Gm. per day concurrently with 2 Gm. of probenecid orally per day, there is no interference with the enhancement effects of probenecid upon penicillemia. The lack of interference of salicylates with probenecid

was observed at circulating plasma concentrations of salicylates approximating 10 mg. per 100 ml. and probenecid concentrations of 5 to 6 mg. per 100 ml. (c) Probenecid plasma concentrations that are pharmacologically active with respect to penicillin have no effect upon salicylemia.

The authors wish to express their indebtedness to Miss JoAnn Binns, Miss Lorraine Leidy, and Miss Sue Paulson, all of the Research Laboratories of Sharp & Dohme Division of Merck & Company, Inc. for technical assistance.

## REFERENCES

1. Beyer, K. H., Wiebelhaus, V. D., Russo, H. F., Peck, H. M., and McKinney, S. E.: 'Benemid'' (p-[Di-n-propylsulfamyl]-Benzoic Acid)—An Anticatabolite: Its Pharmacological Properties, Fed. Proc. **9**: 258, 1950.
2. Russo, H. F., Bunn, C. E. E., Miller, A. K., and Scheidy, S. F.: Comparison of Intramuscular Repository Procaine Penicillin With Oral Penicillin and Probenecid, J. Am. Vet. M. A. **124**: 284-289, 1954.
3. Boger, W. P., Beatty, J. O., Pitts, F. W., and Flippin, H. F.: The Influence of a New Benzoic Acid Derivative on the Metabolism of Para-Aminosalicylic Acid (PAS) and Penicillin, Ann. Int. Med. **33**: 18-31, 1950.
4. Talbott, J. H.: Experimental Observations in Acute and Chronic Gout, Proc. Inst. Med. Chicago **18**: 383-395, 1951.
5. Bishop, C., Rand, R., and Talbott, J. H.: The Effect of ''Benemid'' (p-[Di-n-propylsulfamyl]-Benzoic Acid) on Uric Acid Metabolism in One Normal and One Gouty Subject, J. Clin. Invest. **30**: 889, 1951.
6. Gutman, A. B., and Yu, T. F.: ''Benemid'' (p-[Di-n-propylsulfamyl]-Benzoic Acid) As Uricosuric Agent in Chronic Gouty Arthritis, Tr. A. Am. Physicians **64**: 279-288, 1951.
7. Yu, T. F., and Gutman, A. B.: Mobilization of Gouty Tophi by Protracted Use of Uricosuric Agents, Am. J. Med. **11**: 765-769, 1951.
8. Michaels, Max, Jr.: Personal communication.
9. Pascale, Luke, R., Dubin, A., and Hoffman, W. S.: Therapeutic Value of Probenecid (''Benemid'') in Gout, J. A. M. A. **149**: 1188-1194, 1952.
10. Smith, R. T., Strickland, S. C., Maguire, E. F., and Hermann, I. F.: ''Benemid'' in Gout, 8th Int. Congress on Rheumatic Diseases, 1953, Geneva, Switzerland.
11. Talbott, J. H.: Diagnosis and Treatment of Gouty Arthritis, California Med. **79**: 220-226, 1953.
12. Pitts, F. W., and Chambers, J. S.: The Influence of (p-[Di-n-propylsulfamyl]-Benzoic Acid) on the Excretion of Uric Acid, U. S. Armed Forces M. J. **3**: 1219-1224, 1952.
13. Burke, J. C.: Methods in Medical Research, vol. 1, Chicago, 1948 Yearbook Publishers, Inc.
14. Brodie, B. B., Udenfriend, S., and Coburn, A. F.: The Determination of Salicylic Acid in Plasma, J. Pharmacol. & Exper. Therap. **80**: 114-117, 1944.
15. Tillson, E. K., Schuchardt, G. S., Pitt, A. A., Gass, R. W., and Beyer, K. H.: ''Benemid'' (p-[Di-n-propylsulfamyl]-Benzoic Acid), an Anticatabolite: Its Estimation in Body Fluids, Fed. Proc. **9**: 1950.
16. Boger, W. P., Matteucci, W. V., Schimmel, N. H., and Flippin, H. F.: ''Benemid,'' Its Influence on PAS, Penicillin and Other Antibiotics, Scientific Exhibit, A. M. A., June 11-15, 1951.
17. Talbott, J. H.: Gout and Gouty Arthritis, Modern Medical Monograph, New York, 1953, Grune & Stratton, Inc.
18. Yu, T. F., Sirota, J. H., and Gutman, A. B.: Influence of Plasma Salicylate Concentration on Urate Clearance in Man, Clin. Res. Proc. **1**: 107-108, 1953.
19. Boger, W. P., Gallagher, M. E., and Beatty, J. O., Probenecid and Salicylates: I. Carinamide and Probenecid Compared With Regard to Effect on Salicylemia. To be published.
20. Boger, W. P.: Proc. of A. A. A. S. Conf. on Medicinal Chem., pp. 55-68, Aug. 20-24, 1951.

# EFFECT OF GRADED AMOUNTS OF EPINEPHRINE ON NORMAL AND STRESS LYMPHOCYTES OF INTACT AND ADRENALECTOMIZED MICE

JULES A. FRANK, M.D., AND THOMAS F. DOUGHERTY, PH.D.
SALT LAKE CITY, UTAH

PREVIOUS publications from this laboratory have called attention to two morphologic types of lymphocytes which exist in the normal, intact mouse: normal or typical lymphocytes, having a round nucleus and narrow rim of basophilic cytoplasm, and stress lymphocytes, which are found in fewer numbers and are characterized by cellular distortion and increased amounts of poorly basophilic cytoplasm.[1] Moreover, it has been found that the lymphocytosis occurring in stressed, adrenalectomized mice is due primarily to an increase in numbers of stress lymphocytes.[1] It is postulated that the increased production of stress lymphocytes is due to nonadrenocortically mediated effects of stressors.

Further experiments were designed to determine whether normal and stress lymphocytes respond in a dose-response manner to graded amounts of epinephrine. In addition, measurements of the cell and nuclear diameters of normal and stress lymphocytes were made.

## MATERIALS AND METHODS

Sixty-two male CBA mice, 12 to 16 weeks old, were selected. The body weights ranged between 26 and 28 Gm. Epinephrine HCl (aqueous 1:1,000) was employed as a nonspecific stress stimulus and diluted with normal pyrogen-free saline solution into 0.1 ml. doses of 0.2 γ, 50 γ, and 150 γ.

Adrenalectomy was performed under ether anesthesia by the usual dorsal approach one hour prior to the intraperitoneal injection of epinephrine.

Tail blood samples for total leukocyte counts and differentials were taken one hour prior to epinephrine injection at 10, 30, 60, 120, and 240 minutes after injection. Blood films were stained with May-Grünwald-Giemsa, and differential leukocyte counts performed on a minimum of 200 cells with at least two different observers contributing counts on each animal. Lymphocytes were classified as normal and stress lymphocytes. Cells were measured by a calibrated projector.

The experimental procedures were performed on the following groups of animals: (1) three groups of intact mice given 0.2 γ, 50 γ, and 150 γ of epinephrine, respectively, and (2) three groups of adrenalectomized mice given 0.2 γ, 50 γ, and 150 γ of epinephrine, respectively. Control values consisted of the absolute numbers of the various lymphocytes determined on a group of 25 intact nonstressed mice and a group of 25 nonstressed adrenalectomized mice one hour after operation.

---

From the Department of Anatomy, University of Utah College of Medicine, Salt Lake City, Utah.

Supported in part by a grant from the Department of the Army, Contract #DA-49-007-MD-130.

Received for publication Oct. 21, 1954.

RESULTS

*Intact Mice.*—Lymphocyte counts for intact mice and the lymphocyte responses of groups of mice given 0.2 γ, 50 γ, and 150 γ epinephrine are presented in the upper half of Fig. 1. The bars represent total lymphocytes in thousands, the clear portions the numbers of normal lymphocytes, and the hatched portions the numbers of stress lymphocytes. A lymphopenia primarily due to a decrease in numbers of normal lymphocytes was found at 30 minutes in the 0.2 γ group and at 120 minutes in the 50 γ group. No lymphopenic response occurred in the 150 γ group. A lymphocytosis due to an increase in numbers of stress lymphocytes was found in the 150 γ group, although normal lymphocytes were found to be decreased at all time intervals studied.

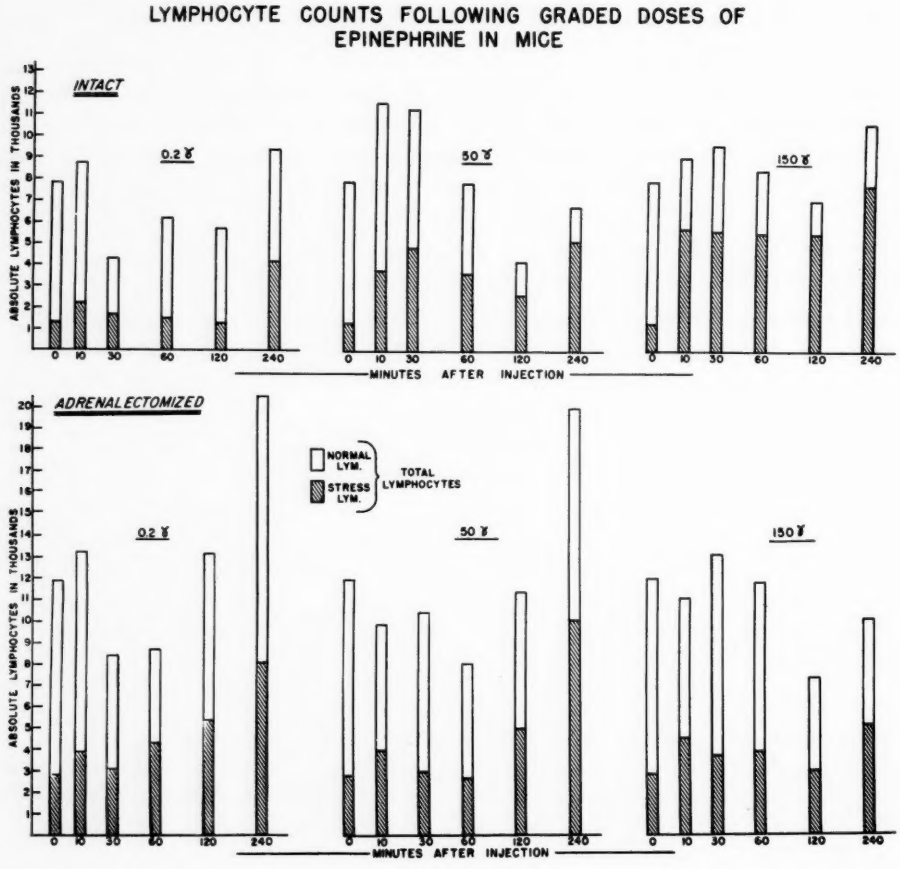



Fig. 1.

As may be seen in Fig. 1, the numbers of stress lymphocytes were increased at 240 minutes in the lowest dose (0.2 γ) and at all time intervals in the 50 γ and 150 γ groups, with the total number of these cells increasing with increasing doses of epinephrine. A dose-response relationship was found in intact mice, i.e., the numbers of stress lymphocytes increased with increasing doses of epinephrine.

*Adrenalectomized Mice.*—The configuration of the total lymphocyte response curves of these animals was found to be similar to that of intact animals (lower half of Fig. 1). However, a lymphocytosis was found in all three groups which was greater than that for the intact mice. Numbers of normal lymphocytes did not vary appreciably in any group until 240 minutes, at which time a marked increase was found for the 0.2 γ and 50 γ groups. Numbers of normal lymphocytes were not altered significantly at any time in the 150 γ group.

Significant increases in numbers of stress lymphocytes were found at 60, 120, and 240 minutes with 0.2 γ, at 120 and 240 minutes with 50 γ, and not until 240 minutes with 150 γ. Thus, differences in dosage did not result in significant differences in response to stress lymphocytes, although there seemed to be a delay in the response with higher dosage. The dose-response relationship of stress lymphocytes found in intact mice was abolished by adrenalectomy.

The cell diameters and areas, the nuclear diameters and areas, and the ratios of cell to nuclear size are presented in Table I. Stress lymphocytes are larger than normal lymphocytes in that the cell diameters, nuclear diameters, and the cell-nuclear ratios are increased.

TABLE I. A COMPARISON OF THE MEASUREMENTS OF NORMAL AND STRESS LYMPHOCYTES

| GROUP | NO. OF MICE | MEAN CELL DIAMETER | MEAN CELL AREA | MEAN NUCLEAR DIAMETER | MEAN NUCLEAR AREA | MEAN RATIO OF CELL TO NUCLEUS |
|---|---|---|---|---|---|---|
| Normal subjects | 10 | 10.69 μ ±.06 | 86.62 μ ±1.02 | 9.47 μ ±.10 | 68.71 μ ±1.61 | 1.306 μ ±.021 |
| Stress | 10 | 11.91 μ ±.13 | 113.18 μ ±2.41 | 10.01 μ ±.10 | 79.21 μ ±1.76 | 1.465 μ ±.038 |

In each case $P < .01$.

## DISCUSSION

It is apparent from the data presented here that the total absolute lymphocyte response of both intact and adrenalectomized mice masks the differential response of normal and stress lymphocytes. In the intact animal normal lymphocytes decreased in number, and stress lymphocytes increased following the nonspecific stress stimulus employed. In adrenalectomized mice there is an increased number of stress lymphocytes with no significant increase in normal lymphocytes until 240 minutes in the 0.2 γ and 50 γ doses.

Second, a relationship exists between the dose and the magnitude of the response of both types of lymphocytes in intact mice, i.e., the greater the stress the higher the number of stress lymphocytes. This relationship was not found in adrenalectomized mice.

The configuration of the lymphocyte curves was similar in both intact and adrenalectomized animals, although all levels were higher in adrenalectomized animals. It must be pointed out that adrenalectomy was performed one hour prior to the injection of epinephrine. Adrenalectomy, in and of itself, is followed by a lymphopenia at one hour and then by a lymphocytosis which, however, does not progressively increase.[1] Therefore, the significance of the lymphocytosis following epinephrine given one hour after adrenalectomy is

further emphasized. A further experiment has been carried out in which five days elapsed between adrenalectomy and injection of epinephrine. No initial lymphocytosis was observed under the latter conditions although the response was characterized by a gradual increase in total and stress lymphocytes at 30, 60, 120, and 240 minutes.

In addition to the previously described morphologic changes which characterize stress lymphocytes, it has been found in these studies that both the nucleus and cytoplasm are significantly increased in size over normal lymphocytes.

The differences in response described previously of intact and adrenalectomized mice to epinephrine have given rise to the hypothesis that stress stimuli exert two effects on blood lymphocytes. One effect is that increased secretion of adrenocortical hormones following stress selectively results in a decrease in number of normal lymphocytes. The other is a nonadrenocortically mediated effect resulting in an increase in number of the morphologically altered stress lymphocytes.

## SUMMARY

Experiments have been performed on both intact and adrenalectomized mice using graded amounts of epinephrine as nonspecific stress stimuli. In intact mice, stress stimuli result in dose responses of numbers and types of lymphocytes. Normal lymphocytes are decreased in number, whereas the number of stress lymphocytes is increased. Adrenalectomy abolishes the graded increases in number of stress lymphocytes following graded doses of epinephrine. The duration of each phase is prolonged with increased doses. Cell and nuclear measurements of stress lymphocytes are significantly larger than those of normal lymphocytes.

## REFERENCE

1. Dougherty, T. F., and Frank, J. A.: The Quantitative and Qualitative Responses of Blood Lymphocytes to Stress Stimuli, J. LAB. & CLIN. MED. **42**: 530-537, 1953.

# *LABORATORY METHODS*

## A CARDIO-ROENTGEN ACTUATOR: AN INSTRUMENT FOR ACTUATING THE ROENTGENOSCOPE IN END SYSTOLE OR END DIASTOLE OF THE HEART ACTION

FRANK W. NOBLE, M.E.E., BERT R. BOONE, M.D., HAROLD T. DODGE, M.D., AND ARMAND E. BRODEUR, M.D.
BETHESDA, MD.

### INTRODUCTION

ROENTGENOGRAMS of the heart and great vessels are routinely taken without reference to the phases of the cardiac cycle. For many purposes such procedure is entirely satisfactory. However, circumstances frequently arise where the clinical investigator desires the roentgenograms to be taken at known ventricular volume phases of the cardiac cycle. So taken, sequential films on the same subject become comparable, changing heart size during treatment can be observed, and physiologic differences at the several phases of the heart cycle may be studied.

A number of such instruments were developed in the past in individual laboratories and x-ray departments, but interest in their application seems to have lagged for one reason or another. Also, at the present time there does not appear to be any such apparatus commercially available.

With these observations in mind and a request on hand, an instrument has been designed and completed which will actuate the roentgenoscope, take film exposures at any desired phase of the cardiac cycle, and place a time marker on the simultaneously recorded electrocardiogram at the point in the heart cycle the film was taken.

### METHODS AND MATERIALS

Numerous articles and physiologic texts are in substantial agreement on the correlation of the phases of the electrocardiographic cycle to the phases of the ventricular volume cycle. The electrocardiographic cycle, being electrical in character, readily amplified, and easily obtained from the intact subject, provides a convenient electrical source from which to select a correlated phase of the ventricular volume which is to be filmed.

The two most usual phases of the ventricular volume during which roentgenograms are desired are in (1) end diastole and (2) end systole. The R-S limb of the electrocardiogram R wave occurs during end diastole, so that a film exposed in this period would portray the ventricles in a filled state, i.e., end diastole. The peak of the R wave is utilized by the apparatus to be described, to trigger the roentgenoscope for the x-ray exposure of the end-diastolic film. The film exposure occurs about 0.01 second after the peak of the R wave and is of short duration.

The end of the ECG T wave coincides with end systole, the closure of the semilunar valves, and onset of isometric relaxation. A film exposed at the end of the T wave or late on its downslope would portray the ventricles in an emptied state, i.e., end systole. Again the peak of the R wave provides the initial signal source, which then passes through a proper time delay circuit and triggers the roentgenoscope at a time corresponding to end

From the Laboratory of Technical Development, National Heart Institute, National Institutes of Health, Public Health Service, Bethesda, Md.
Received for publication Oct. 8, 1954.

systole. Since the time duration of the cardiac cycle varies inversely with the heart rate, a series of appropriate time delays corresponding to heart rate must be incorporated in the apparatus. A multiple-tapped switch is provided, each tap selecting a different delay circuit, with the switch knob calibrated in terms of heart rate. Thus, the subject's heart rate can be counted, the knob turned to that heart rate, and the proper delay will have been selected to trigger the roentgenoscope at end systole.

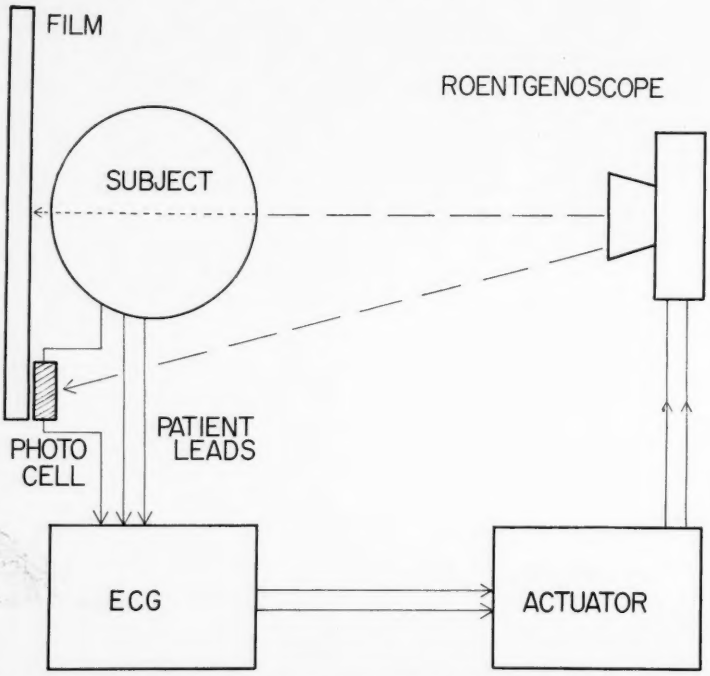



Fig. 1.—A block diagram of the components of the cardio-roentgen actuator. (*See text.*)

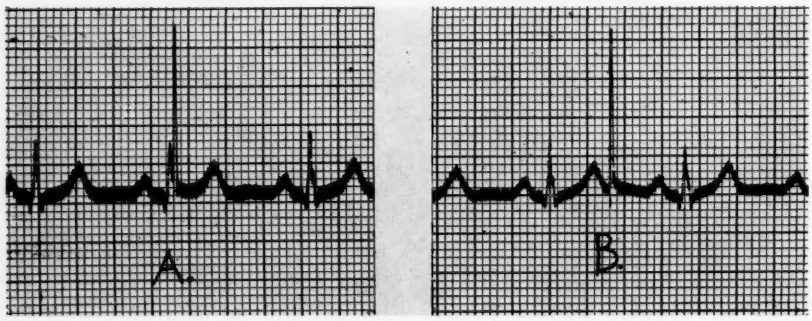



Fig. 2.—*A*, The tall spike on the down-limb of the R wave indicates when the radiograph Fig. 3 was taken. *B*, The spike at the end of the T wave indicates when radiograph Fig. 4 was taken.

Some variability of correlation at this phase in the cardiac cycle between the electrical and mechanical events may be shown to exist, particularly under the influence of various therapeutic agents. This situation can be compensated for, when necessary, by varying the selection of the time delay circuits and observing the timing spike on the ECG.

The operation of the instrument may best be explained with reference to the block diagram, Fig. 1. The photocell has been made sensitive to x-ray by attaching to its face

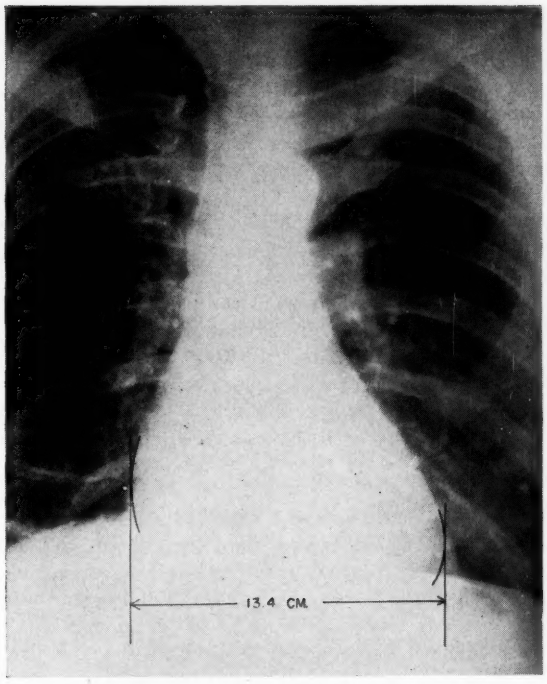



Fig. 3.—An anteroposterior radiograph of the heart taken in end diastole as shown by the ECG time record Fig. 2*A*. Transverse width 13.4 cm.

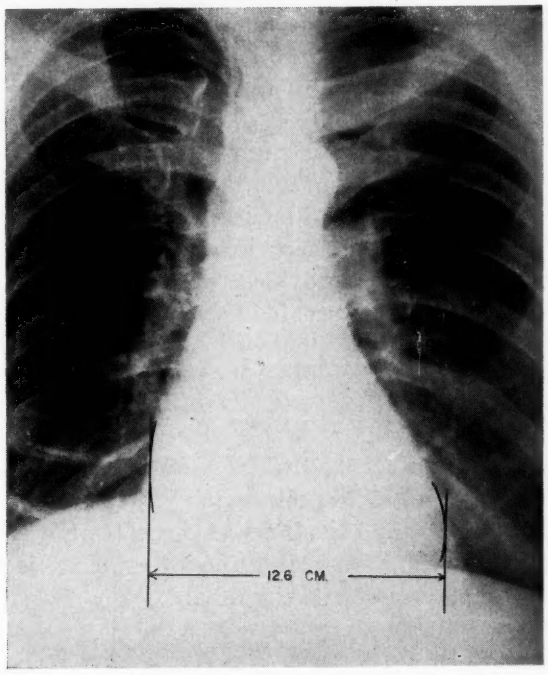



Fig. 4.—An anteroposterior radiograph of the heart taken in end systole as shown by the ECG time record Fig. 2*B*. Transverse width 12.6 cm.

a piece of Patterson B-2 fluoroscopic screen. The cell is placed in the field of the x-ray beam and connected in series with one of the active electrocardiograph leads. Patient Lead 2 has been most frequently used. Thus, when the x-ray machine is operated, the photocell output adds to the electrocardiograph potential at the input to the ECG, producing a short pulse superimposed upon the normal ECG record indicating the time of exposure. The ECG is a standard direct writing machine with a hot stylus pen. The ECG potential is taken from one side of the pen to the input of a calibrated delay circuit. This circuit accepts only the peak of the R wave of the ECG, delays a preset period of time, then closes a relay which in turn actuates the x-ray machine. The x-rays impinge upon the photocell and produce a marker pulse on the ECG record as explained previously.

The ECG records are of the type given in Fig. 2. *A* shows the x-ray impulse at minimum delay (approximately 0.01 sec.) following the peak of the R wave. The radiograph, Fig. 3, resulting from this exposure, therefore, shows the heart at end diastole. The transverse width is 13.4 cm. *B*, in Fig. 2, shows the x-ray impulse suitably delayed with respect to the peak of the R wave so that the radiograph, Fig. 4, resulting from this exposure shows the heart at end systole. Here, the transverse width is 12.6 cm. The difference in heart size on the x-ray plates is evident from the dimensions given on the figures, i.e., 8 mm. The true dimensions of the heart may be found by a simple calculation based upon the geometry of the system.

Additional features of the actuator are a neon indicator panel light which flashes on at the peak of the R wave and off at the end of the delay interval, and a panel push button to be pressed when the operator has completed instructions and arrangements with the patient. Following the pressing of the push button, the actuator will trigger the roentgenoscope after the next R wave and then inactivate itself, thus avoiding multiple exposures. The film cassette may be quickly changed, the actuator dial set for the next phase, and the next film taken.*

## DISCUSSION

The particular type of delay circuit incorporated in the design is the simplest but not the most accurate circuit available. The considerable variability in the time between beats of a typical heart renders the use of a highly precise and complex delay network of questionable value. Furthermore, the provision of a marking pulse on the ECG indicating the exact time of exposure prevents errors. If the exposure was slightly out of time, a longer or shorter delay may be switched in and another film taken.

In the event that it is desired to make exposures at delay times different from those already incorporated in the switch, any delay time from 0.01 second up to several heart cycles may be provided by attaching an external capacitor decade box to a pair of terminals provided at the rear of the instrument.

A possible application of the photocell marker is in the field of angiocardiography where the exact time of exposure of each plate is desired.

## SUMMARY

An apparatus has been described which allows radiographs to be made at any phase of the heart cycle. The peak of the R wave of the ECG is used to trigger a calibrated time delay circuit which actuates the roentgenoscope at the end of the delay interval. The exact time of exposure is presented in the form of a short pulse superimposed on the ECG.

*The detailed circuit diagrams, component parts list, and calibrating data will be supplied upon written request to the author.

# AN ANESTHESIA-BRONCHOSPIROMETER VALVE

PAUL O. ANDERSON, M.D.,* AND JOHN J. CINCOTTI, M.D.**
RUTLAND HEIGHTS, MASS.

A STUDY of individual lung participation from the period immediately preceding the induction of general anesthesia and discontinuously throughout thoracic surgical procedures has been carried out.[1] This approach permits the investigation of (a) the effects produced on isolated lung function during steady-state anesthesia by the gamut of general thoracic surgical procedures and (b) the methods of management which influence favorably, or unfavorably, the end-operative functional status of the patient. During this study the need for easy rapid switching from the anesthetic, bronchospirometric, or suction circuits for either lung led to the construction of a simple, light, easily cleaned, leak-proof, four-way valve which can be sterilized by autoclaving. All orifices of the valve have internal diameters of 7 mm.

Two such valves are used side by side with each valve permitting control of one lung, independent of the other. Fig. 1 shows the assembled Carlen's double-lumen tube and attached valves with a suction catheter (12 French plastic ureteral) extending through the entire length of the lumen for the right lung, while the valve for the left lung is set for either anesthesia or spirometer tracing. There are two parts to each valve, labeled "A" and "B." The diagram (Fig. 2) shows a single valve partly cut away. The single connecting tube of part "A" fits into one lumen of a double-lumen bronchial catheter (Carlen's), while the connecting tubes of part "B" are connected to one bell of a twin spirometer and to the anesthesia machine. Between these connecting tubes is the suction orifice. Part "B" slides within the slot of "A" so that three circuits are available to either lung. Stops (not shown in the diagrams) are provided for each valve so that for each desired setting of the valve instant and exact alignment of the lumens of parts "A" and "B" is assured.

Each valve† (parts "A" and "B" together) weighs approximately 50 grams. The valve, which can be operated by slight finger pressure, will not shift after once being set until it is again moved by hand. The valve does not leak at pressure of 80 p.s.i., which is far above those pressures that can be used safely. The seal is attained with the use of plastic "O" rings seated between the moving parts of the valve. Wear is insignificant; no leakage will develop after prolonged use. The "O" rings can be easily and cheaply replaced and withstand autoclaving and other sterilizing procedures. The tubes projecting from the valve are designed to fit within the Carlen's double-lumen bronchial

Received for publication Oct. 18, 1954.

*Present address: VA Hospital, Newington, Conn.; formerly from the Department of Medicine and Pulmonary Function Laboratory, VA Hospital, Rutland Heights, Mass.

**Chief, Surgical Service, VA Hospital, Brooklyn, N. Y., and Associate Clinical Professor of Surgery, State University of New York, College of Medicine at New York, Brooklyn, N. Y.; formerly Chief, Surgical Service, VA Hospital, Rutland Heights, Mass.

†Available through the Worcester Taper Pin Company, Worcester, Mass.

catheter. The flow-resistance characteristics of the airway within the valve are the same as for the Carlen's catheter; no additional resistance to ventilation of the lungs will result through use of the valve. In fact, since the valve eliminates the need for extra lengths of tubing, it will indirectly aid ventilation.[2]

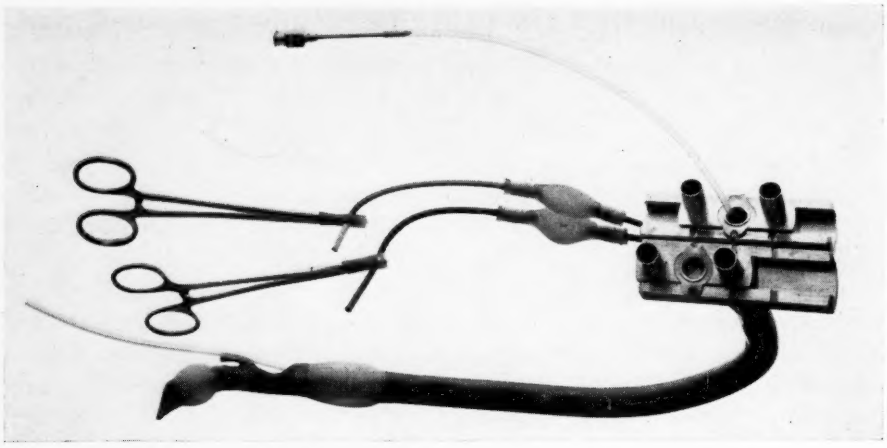


Fig. 1.—Assembled Carlen's double-lumen tube and attached valves.

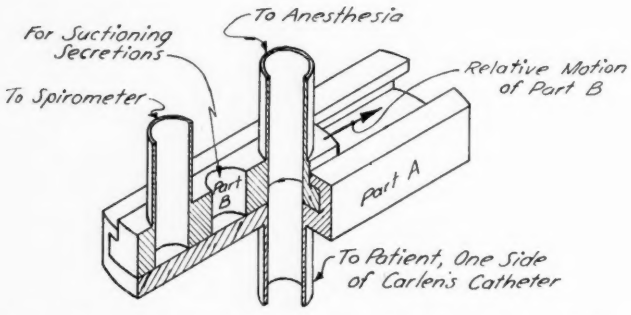



Fig. 2.—A diagram of a single valve. Part section of assembled valve in position for anesthesia.

This anesthesia bronchospirometer valve affords a convenient method for the study of individual lung function during anesthesia and surgical procedures. Spirometer tracings ensure that isolation, in fact, obtains and additionally serves as a discontinuous record of individual lung tidal and minute-volume ventilation and oxygen uptake. Relaxation volumes and mid-position changes may be recorded. The suction orifice permits unhurried, unilateral evacuation of bronchial secretions.

REFERENCES

1. Cincotti, J. J., Watson, R. T., and Gaensler, E. A.: Unpublished data.
2. Gaensler, E. A., Maloney, J. V., and Björk, V. O.: Bronchospirometry. II. Experimental Observations and Theoretical Considerations of Resistance Breathing, J. LAB. & CLIN. MED. 39: 935, 1952.

# SIMPLE APPARATUS FOR CONCENTRATING BIOLOGIC FLUIDS OF LOW PROTEIN CONTENT

CHARLES H. GROGAN* PH.D., AND ELIZABETH ROBOZ,** PH.D.
WASHINGTON, D. C.

## MATERIALS AND METHODS

PAPER electrophoresis provides us with a very efficient technique for the separation of proteins by utilizing the differences in their electrical mobilities. Although it is a micromethod, a certain minimum quantity of the proteins is needed for an effective resolution and staining on the paper. A volume that can be applied conveniently is about 0.01 to 0.03 ml., and this should contain 1.0 to 1.5 mg. proteins. If a solution that has a very low protein concentration—such as cerebrospinal fluid—is measured, it has to be concentrated 25 to 100 times, depending on its protein content. Among the methods used for concentration[1-3] is dialysis against high molecular weight compounds such as dextran[4] and polyvinylpyrrolidone.[5] It has been reported[6] that 2 to 7 days are necessary to concentrate the cerebrospinal fluid to the desired volume; if this is carried out at room temperature, it is difficult to avoid mold or bacterial growth, especially if dextran is used.

The present paper describes a simple experimental arrangement whereby the dialysis is carried out in the refrigerator at 5° in about 16 to 20 hours for fluids containing less than approximately 25 mg. of protein per 100 ml. If higher concentrations of protein are present, the time necessary to concentrate the fluid sufficiently for paper electrophoretic separation is less. The process, on the scale necessary to prepare protein samples from 10 to 20 ml. of urine, cerebrospinal fluid, etc., for paper electrophoresis, involves stirring the dextran† or polyvinylpyrrolidone‡ solutions outside the dialysis membrane with small glass-covered magnetic stirrers.

The very simple and effective apparatus employed, constructed from 5 by 10 cm. thick-walled specimen bottles, a No. 9 rubber stopper, and glass tubing, is shown with approximate dimensions in Fig. 1. When Visking 9/16″ inflated diameter seamless Cellophane tubing was employed, 10 to 12 ml. of protein solution could be dialyzed against 100 to 125 ml. of polymer solution. Four such arrangements can be conveniently run simultaneously in a 9 cubic foot refrigerator, employing the shelves near the freezing coils, at a temperature between 5° to 8° C., as illustrated in Fig. 2. With a larger cold compartment or cold room more bottles, or conceivably larger scale concentrations, could be handled.

The relative effectiveness of the dextran preparation and the "Plasdone" was tested by a series of experiments on replicate samples with and without stirring. The polymers were used at a 15 per cent (w/v) concentration. Ten milliliters of the protein-containing fluids were dialyzed against 100 ml. of polymer solution. Typical results obtained with pathologic urines and cerebrospinal fluids are shown in Table I. The results show that the stirring effects at least a tenfold increase in efficiency of concentration with cerebro-

This investigation was supported in part by a grant from the United States Public Health Service.

Received for publication Nov. 1, 1954.

*Environmental Cancer Section, National Cancer Institute, Department of Health, Education, and Welfare, Bethesda, Md.

**Departments of Biological Chemistry and Neurology, Georgetown University School of Medicine, Washington, D. C.

†Dextran, clinical, average molecular weight 70,000, Commercial Solvents Corp., Terre Haute, Ind.

‡"Plasdone," polyvinylpyrrolidone, General Aniline and Film Corp., New York, N. Y.

spinal fluid and a fourfold to sixfold increase with urine. Data for the final volumes are not completely indicative of the relative efficiencies, however, because, as the internal volume becomes smaller, the rate of concentration slows down markedly without stirring.

In order to determine whether small molecular weight compounds present as impurities in the commercial "Plasdone" and dextran might diffuse into the dialyzing solution and contaminate the protein, experiments were carried out in which 10 ml. portions of 0.9 per cent saline were dialyzed against dextran and "Plasdone." These experiments also served to compare the relative effectiveness of the two polymar preparations for concentrating protein-containing biological fluids. The results are shown in Table II. The "Plasdone" preparation was more effective than the dextran preparation on a weight basis. When "Plasdone" was increased to 25 per cent (w/v), only 7 hours were needed for the concentration of 10 ml. of cerebrospinal fluid.

The concentrate was removed from the dialysis bag and analyzed for polyvinylpyrrolidone by the method of Levy and Fergus[7] or for dextran by the method of Roe.[8] Although small amounts of polymer were found in the concentrate in all cases, the electrophoresis patterns were unaffected when compared with the patterns obtained from the same fluid samples from which the proteins were concentrated by other techniques.[3]

Dialysis apparatus, such as described by Reiner and Fenichel,[9] which employs internal stirring is unsuitable because, as the internal volume decreases, the membrane collapses around the stirrer.

The apparatus and technique described herein for concentrating biologic fluids of low protein content have the advantage that they effect a rapid concentration without denaturation at a low temperature, avoid growth of microorganisms, and can be readily carried out under sterile conditions. We believe that this method can be used for concentrating other biologic fluids such as ascitic and cyst fluids and also enzyme-containing solutions.

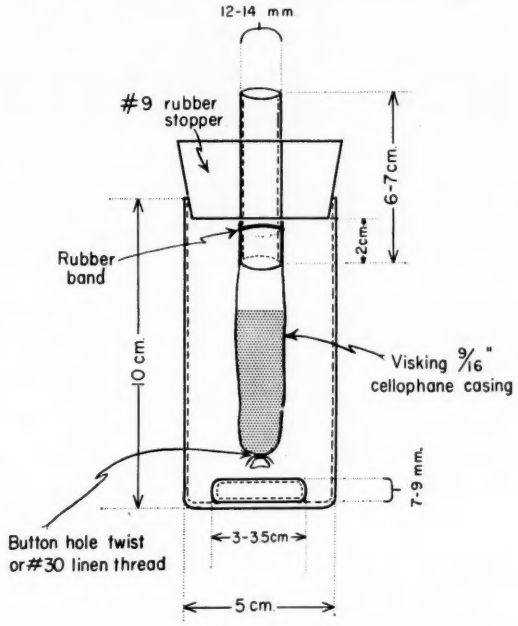
TABLE I. CONCENTRATION OF CEREBROSPINAL FLUID AND PATHOLOGIC URINES BY DIALYSIS AGAINST DEXTRAN AND "PLASDONE"

| MATERIAL | CONDITIONS | VOLUME OF CONCENTRATE (ML.) | TIME (HR.) | POLYMER |
|---|---|---|---|---|
| C. S. Fluid | Stirred | 0.34 | 18 | PVP |
| C. S. Fluid | Stirred | 0.20 | 18 | PVP |
| C. S. Fluid | Not stirred | 2.6 | 18 | PVP* |
| C. S. Fluid | Not stirred | 2.8 | 18 | PVP* |
| Urine | Stirred | 0.75 | 20 | PVP |
| Urine | Stirred | 0.90 | 20 | PVP |
| Urine | Not stirred | 3.4 | 20 | PVP |
| Urine | Not stirred | 3.9 | 20 | PVP |
| C. S. Fluid | Stirred | 4.7 | 20 | Dextran |
| C. S. Fluid | Not stirred | 7.2 | 20 | Dextran |

*When left an additional 24 hours for a total time of 42 hours, both had volumes of approximately 0.5 ml.

TABLE II. COMPARISON OF DEXTRAN AND "PLASDONE" ON DIALYSIS VS. 0.9 PER CENT SALINE

| CONDITIONS | VOLUME OF CONCENTRATE (ML.) | TIME (HR.) | MATERIAL | CONTENT OF POLYMER IN CONCENTRATE (μG.) |
|---|---|---|---|---|
| Stirred | 0.06 | 20 | PVP | 25-30 |
| Stirred | 0.08 | 20 | PVP | 22-26 |
| Not stirred | 1.40 | 24 | PVP | 350 |
| Not stirred | 1.30 | 24 | PVP | 280 |
| Stirred | 1.20 | 24 | Dextran | 310 |
| Stirred | 1.30 | 24 | Dextran | 340 |
| Not stirred | 3.6 | 24 | Dextran | 730 |
| Not stirred | 3.6 | 24 | Dextran | 680 |




DIALYZER

Fig. 1.

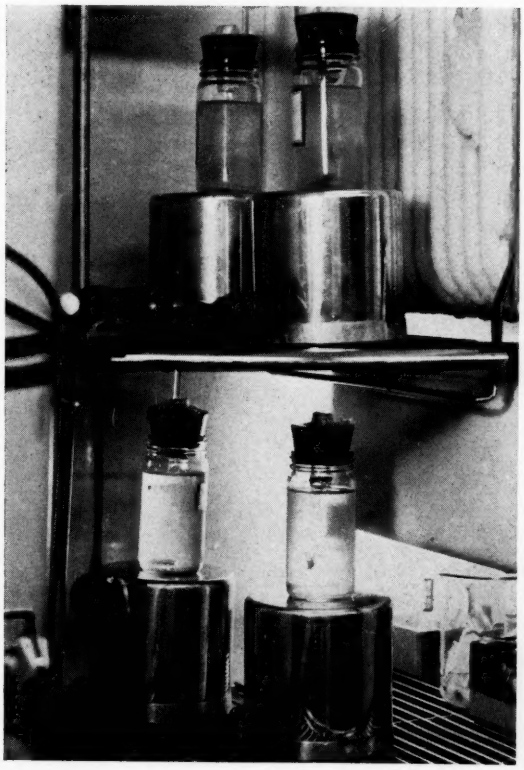


Fig. 2.

REFERENCES

1. Bucher, T., Matzelt, D., and Pette, D.: Paper Electrophoresis of Cerebrospinal Fluid, Klin. Wchnschr. **30**: 325, 1952.
2. Eaton, J. C., and Gardner, M. D.: Separation of Cerebrospinal Fluid Proteins by Paper Electrophoresis, Biochem. J. **55**: xxv, 1953.
3. Roboz, E., Hess, W. C., and Temple, D.: Paper Electrophoretic Estimation of Proteins in Cerebrospinal Fluid, J. LAB. & CLIN. MED. **43**: 789, 1954.
4. Schneider, G., and Wallenius, G.: Electrophoretic Studies on Cerebrospinal Fluid Proteins, Scandinav. J. Clin. & Lab. Invest. **3**: 140, 1951.
5. Cumings, J. N.: The Examination of the Cerebrospinal Fluid and Cerebral Cyst Fluid by Paper Strip Electrotrophoresis, J. Neurol., Neurosurg. & Psychiat. **16**: 152, 1953.
6. Esser, H., Heinzler, F., and Wild, H.: A Simple Method for Protein Concentration of the Cerebrospinal Fluid in Preparation for Paper Electrophoresis, Klin. Wchnschr. **30**: 228, 1952.
7. Levy, G. B., and Fergus, D.: Microdetermination of Polyvinylpyrrolidone in Aqueous Solution and in Body Fluids, Anal. Chem. **25**: 1,408, 1953.
8. Roe, J. H.: The Determination of Dextran in Blood and Urine With Anthrone Reagent, J. Biol. Chem. **208**: 889, 1954.
9. Reiner, M., and Fenichel, R. L.: Dialysis of Protein Solutions for Electrophoresis, Science **108**: 164, 1948.